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Robert S Kerr Environmental Research Lab, Ada, OK

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(Volume II Microbiological Implications)

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16. ABSTRACT

The city of San Angelo, Texas, has been using agricultural land for decades as a means of disposing of all of its municipal sewage after primary treatment. Water applications have been high enough to satisfy crop requirements for a 600 ha farm even though the farm consists of only 259 ha. The farm routinely supports about 500 cattle on its pastures and produces both row and hay crops. Land application of sewage has public health implications, and this study was conducted to evaluate these concerns. This was accomplished by monitoring the soils and waters on the farm to determine the incidence of Salmonella and parasites. Salmonella was isolated from various locations on the farm but the frequency of isolation was not unusually high. Possible human parasites were not found in any effluent but were present in the sludge in holding lagoons. The parasite population in cattle on the farm did not increase during the months the cattle were monitored. There was an unusually high population of animal parasites in the soils as compared to off-farm control soils. This is thought to be due to the higher animal density, the vegetative cover, and relatively moist soil conditions on the farm. Column studies using soil from the farm indicated viruses could be leached through the soils. Their potential health hazard could not be determined due to insensitive detection techniques.

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VOLUME II

MICROBIOLOGICAL IMPLICATIONS

by

R. W. Weaver, N. O. Dronen, B. G. Foster, F. C. Heck, and R. C. Fehrmann
Texas A&M University
College Station, Texas 77843

Grant No. R803281

Project Officer

Lowell E. Leach
Wastewater Management Branch
Robert S. Kerr Environmental Research Laboratory
Ada, Oklahoma 74820

ROBERT S. KERR ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ADA, OKLAHOMA 74820

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FOREWORD

The Environmental Protection Agency was established to coordinate administration of the major Federal programs designed to protect the quality of our environment.

An important part of the agency's effort involves the search for information about environmental problems, management techniques and new technologies through which optimum use of the nation's land and water resources can be assured and the threat pollution poses to the welfare of the American people can be minimized.

EPA's Office of Research and Development conducts this search through a nationwide network of research facilities.

As one of these facilities, the Robert S. Kerr Environmental Research Laboratory is responsible for the management of programs to: (a) investigate the nature, transport, fate and management of pollutants in groundwater; (b) develop and demonstrate methods for treating wastewaters with soil and other natural systems; (c) develop and demonstrate pollution control technologies for irrigation return flows; (d) develop and demonstrate pollution control technologies for animal production wastes; (e) develop and demonstrate technologies to prevent, control or abate pollution from the petroleum refining and petrochemical industries; and (f) develop and demonstrate technologies to manage pollution resulting from combinations of industrial wastewaters or industrial/municipal wastewaters.

This report contributes to the knowledge essential if the EPA is to meet the requirements of environmental laws that it establish and enforce pollution control standards which are reasonable, cost effective and provide adequate protection for the American public.

William C. Galegar

William C. Galegar
Director
Robert S. Kerr Environmental Research Laboratory

ABSTRACT

The city of San Angelo, Texas, has been using agricultural land for decades as a means of disposing of all its municipal sewage. The sewage has only received primary treatment. Application rates have been such that both row and hay crops have been grown. Additionally, the farm has routinely supported approximately 500 cattle on its pastures. Even though the farm consists of 259 ha, enough sewage effluent is applied to it to satisfy the water requirements of crops grown on more than 600 ha. Land application of sewage has public health implications. This study was conducted to determine the public health implications of operation of the sewage farm. This was accomplished by monitoring the soils and waters on the farm to determine the incidence of salmonella and parasites. Salmonella was isolated from various locations on the farm but the frequency of isolation was not unusually high. Cattle grazing the pastures on the sewage farm, generally, were not excreting salmonella in their manure. Possible human parasites were not found in any effluent but were present in sludge. The parasite population in cattle on the sewage farm did not increase during the months the cattle were monitored. There was an unusually high population of animal parasites in the sewage farm soils as compared to similar soils off the sewage farm. This was thought to be due to the higher animal density on the farm, the vegetative cover on the farm and the relatively moist soil conditions on the farm. Column studies using soil from the farm indicated viruses could be leached through the sewage farm soils. Methods used for detection of viruses on the sewage farm were not sensitive enough to evaluate their potential health hazard. The only public health hazard that was readily apparent from this study was that seepage creeks originating from effluent applied to the farm contained unusually high populations of coliforms. The seepage water drains into the Concho River, which is used for recreational activities and as a source of drinking water for cities downstream from San Angelo. This problem could be alleviated by expanding the size of the sewage farm so that only enough water is applied to meet crop needs. Presently, most of the applied water leaves the farm as seepage.

This report was submitted in fulfillment of Grant No. R803281 by Texas A&M University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period April 28, 1975, to June 27, 1977, and work was completed as of June 27, 1977.

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SECTION 1

INTRODUCTION

The interest in land disposal of wastes from animals, industries, and municipalities has grown rapidly within the past few years. Rigorous standards discouraging discharge of sewage effluents into waterways have contributed to the current interest in land disposal. The benefits of increasing ground water recharge and increasing soil fertility have also been factors in the widespread appeal of land disposal (Sanitary Engineering Research Lab. 1955). The application of waste water to land has been viewed as an economical treatment process in which the soil acts as a "living filter" (Kardos 1967). But, the application of wastes onto land does not offer a solution to all problems of waste disposal. There are inherent health hazards.

Disease caused by pathogenic microorganisms known to exist in municipal wastes is of primary importance to public health (Krone 1968). The potential for disease transmission by application of sewage to crops has long been recognized (Benarde 1973 and Decker and Steele 1966) and depends on available vectors of infection. Important vectors of infection associated with land disposal of sewage are runoff and deep percolation of water.

Salmonellosis is a disease of major public concern. More than 200,000 cases of salmonellosis are reported annually, but between 1 and 2 million people are actually infected with salmonella annually (Aserkoff, et al. 1970). More than half the cases have been sporadic, but the remaining cases have been associated with epidemics that can usually be traced to contaminated foods of animal origin or to contaminated waters (Steele 1968).

Animal wastes are an important factor in perpetuating and extending the prevalence of salmonella. However, the transport and survival of salmonella in sewage waters is also important (Caldwell 1938, Claudon et al. 1972, Dunlop 1968, Hibbs and Foltz 1969, Kampelmacher and Van Noorle Jansen 1970, Krone 1968, Moore 1971 and World Health Org. 1975). Individuals infected with salmonella excrete an enormous number of cells daily; as many as 10^{11} cells. Therefore, a tremendous inoculum is provided from one infected individual for contamination of water. For example, an inoculum of 10^{11} cells in an 18.9 million liter, or 5 million gallon a day sewage plant, such as the one in San Angelo, Texas, would contain 5 organisms per ml. Only one organism need be ingested under optimal conditions to cause disease. The normal minimal infective dose for salmonella is approximately 10,000 (Moore 1971).

A bacteriological survey was performed on the sewage treatment facility at San Angelo, Texas for 12 months. This facility utilized lagoons for sewage treatment and storage. Water from the lagoons was used to irrigate row crops and forages. Some forages were used for hay but others were grazed by cattle.

Because land disposal is a popular method of sewage treatment in the western areas of Texas, the potential health hazard from this method of sewage treatment was determined. This was accomplished by making parasitological and microbiological measurements on the sewage farm soil, on the river bordering the farm, on seepage creeks entering the river from the farm, and on the cattle grazing on the farm. Groups of bacteria assayed for included coliforms, fecal coliforms, Pseudomonas aeruginosa, and fecal streptococci. Salmonella sp. were qualitatively studied by using an enrichment technique. Also, laboratory experiments were used to gain information on the leaching of bacteria and viruses through soil.

SECTION 2

SUMMARY AND CONCLUSIONS

MICROBIOLOGY

The population of bacteria in the sewage lagoons on the San Angelo sewage farm was only 10% as large as the population in the raw sewage. Total coliforms and fecal coliforms in the seepage creeks fluctuated from month to month between 10 and 1,000 per ml. These populations of coliforms were very high for normal seepage creeks. The ratio of fecal coliforms to enterococci indicated the seepage waters were mainly polluted with sewage and not cattle manure. Generally, the Concho River contained fewer than 50 total coliforms per ml and there was no indication that the population of coliforms in the river was significantly increased by the sewage farm. This was probably due to the large dilution by the river water. **Salmonella** was isolated from raw sewage, from soil on the sewage farm, and from the seepage creeks. The proportion of cattle grazing on the sewage farm that were shedding salmonella in their manure was not unusually high. Apparently, grazing cattle on the sewage farm did not result in their becoming infected with salmonella. The sewage farm had no affect on the microbiological content of deep wells used for drinking water in the area surrounding the sewage farm. Coliform populations in the surface horizon of sewage farm soils ranged from fewer than 100 to 70,000 per g of soil. Similar soils off the sewage farm contained fewer than 100 per g of soil. The highest population of coliforms was in the surface horizon of the sewage farm soils. The number was reduced by more than 90% at 5 cm below the surface of the Rio Concho soil. In laboratory investigations, the San Angelo soil had a greater adsorbtive capacity for a strain of S. typhimurium than for a strain of E. coli. The concentration of S. typhimurium and S. enteriditis in the leachate from a 3 cm soil column was reduced by more than 99%. However, the population of E. coli was reduced by 99.9%. The leachability of all the bacteria was similar for longer columns.

Viruses were not isolated from samples of sewage, soil or water collected from the San Angelo sewage farm. The method used in this study required at least 10 viruses per g of material for detection. Laboratory studies revealed that viruses could be leached through 15 cm columns of the San Angelo clay loam. When either distilled water or physiological saline was used as the eluent, viruses were leached through some columns of this soil. Viruses were not leached through every soil column. The San Angelo clay loam was more effective in adsorbing or inactivating viruses than a Houston Black clay.

PARASITOLOGY

Human parasites were present in sewage entering the San Angelo sewage treatment facility. They were equally prevalent during the winter and summer months. Yet, parasites were rarely detected in irrigation effluent from sewage lagoons. Apparently the parasites settled with the sludge in the lagoons. Sludge in abandoned sludge lagoons contained organisms that appeared to be human parasites. From these observations, we concluded that sewage effluent undergoing a simple pretreatment like settling would be relatively free of parasites. But the sludge would likely contain human parasites and would require special considerations for land application. Inspection of cattle and sheep from the sewage farm showed that these animals were not picking up human parasites and therefore did not serve as reservoirs for them. A Trichuris sp. (nematode) and an Entamoeba sp. (protozoan), which were similar to those found in humans, were found in very low frequencies, but they were presumed to be non-human parasites. There were greater densities of soil-borne larval nematodes of livestock on the farm as compared to a nearby control area. It is probable that continued irrigation of this land and the ability of the treated land to support more vegetation and livestock was beneficial to the survival of these larvae. There was a higher density of nematode eggs in feces from cattle and sheep on the sewage farm compared to livestock off the farm, but parasites in cattle released on the farm did not increase. The parasite population in the cattle when placed on the farm was already comparable to cattle being grazed on the farm. Attempts to reduce the parasite population by using an antihelminthic drug was unsuccessful. The main influence on animal parasites from irrigation of land with sewage water appears to be the indirect effect of extending the survival and density of livestock parasites by keeping the soil moist and providing more vegetation to support a greater animal density which directly increases problems associated with parasites.

SECTION 3

RECOMMENDATIONS

Land application as the method of disposing of the sewage from San Angelo should be continued. The semiarid and warm climate and relatively permeable soils of the San Angelo area are ideal for utilizing land disposal of sewage. Also, farmland is abundant in the area around San Angelo and surface supplies of irrigation water are not readily available. There was no evidence that human or animal health was endangered on the sewage farm due to application of sewage materials. Animal parasites were more abundant on the sewage farm than in surrounding land but this may have been due to the higher animal density on the farm. The sludge deserves special considerations before land disposal because it likely contains most of the human parasites. The main problem with the past procedure of sewage effluent disposal on the sewage farm was lack of sufficient land. This resulted in large quantities of water leaving the sewage farm as seepage. This seepage water frequently contained thousands of coliform bacteria per ml. The sewage farm should be doubled or tripled in size to allow for sufficient land area to adequately utilize the effluent and to prevent sewage bacteria from leaving the farm in seepage water.

SECTION 4

PRESENT STATUS OF INFORMATION

MICROBIOLOGICAL STUDIES

Leaching of Bacteria through Soils

The movement of bacteria through soils has important agricultural and ecological implications. The realization that bacteria travel through soils has been known for many years. In 1909, Dittlorn and Luerssen studied the passage of bacteria through soils. During the 1930's a number of investigations dealt with contamination and bacterial movement from bored hole latrines. Caldwell (1938) and Caldwell and Parr (1937) observed that bacterial travel was a function of the characteristics of the medium, and that accumulations of solids and slimes in the medium slowed bacterial movement.

There has been considerable research conducted on the movement of coliform bacteria through soils from sewage and related wastes. Krone, Orlob, and Hodgkinson, in 1958, concluded that removal of bacteria from water passing through soils was due in part to sedimentation in the pores. They also determined that the sedimentation removal mechanism controlled the subsequent passage of bacteria and that the soil surface played a dominant role in the travel of bacteria, operating in a manner to restrict continued infiltration of bacteria.

Jones (1968) studied the movement of coliform bacteria and organic matter in an aquifer that was artificially recharged with playa lake water. He concluded that the coliform bacteria were not likely to travel more than 31 meters in the fine sand of the aquifer, but bacterial travel might increase in more permeable soils.

In a study by Randall (1970) a municipal well was found to be contaminated with enteric bacteria after 19 years of trouble free operation. It was found that bacteria from the sewage-polluted Susquehana River leached 55 meters to the municipal well after the river bed was excavated. This excavation of the river bed favored induced infiltration by the contaminated river water.

Reneau, et al. (1975) studied the movement of bacteria from septic tank sources in Virginia. Their conclusions were: Coliform bacteria would probably not move into the groundwater system because of restrictive layers of soil, and the drainage water from the watershed would improve with distance from the pollution source as a result of dilution, sedimentation, and bacterial die-off.

Evans and Owens (1972 and 1973) studied factors which affected the concentration of fecal bacteria in land drainage water. Additions of pig manure increased the concentrations of Escherichia coli and enterococci in the drainage water. The flow rate of the discharge also affected the concentration of bacteria in the drainage water.

Korkman (1971) studied the survival and leaching of enterococci from applied liquid manure under field conditions. A field was topdressed with 50 metric tons/ha of liquid pig manure and irrigated with 100 mm of water. Discharge pipes were 1 m below the surface. He reported that 3 percent of the applied bacteria leached into the drainage pipes, and that the bacteria did pass through the pores of clay soils.

There has been very little documentation of the movement of salmonella in soils (Decker and Steele 1966). This has been because the number of salmonella in sewage is much less than the number of coliform bacteria (Boring 1971, Krone 1968, Moore 1971, Dunlop 1968, Water Quality Criteria 1972), and the detection of salmonella in the environment is much more difficult than the detection of coliforms. Yet salmonella has been found in many water supplies throughout the United States (Claudon et al. 1972, Hibbs and Foltz 1964, Thompson et al. 1975). A large waterborne outbreak occurred in Riverside, California that affected over 16,000 individuals. Boring, et al. (1971) investigated the presence of salmonella in municipal wells during the epidemic. They found five or six municipal wells contaminated with Salmonella typhimurium. Water samples from around the city demonstrated 10 times as many salmonella as E. coli. The source of contamination was not known, however there was speculation that the water table may have been contaminated by seepage hundreds of kilometers away from the city.

Control of Bacterial Leaching through Soils

There are a number of factors which control the leaching of bacteria through soils. Among these are filtration, the adsorptive capacity of the bacteria to soil, soil water content, and soil water flux (Bitton, et al. 1974, Burges 1950, Griffin and Quail 1968, Hattori and Hattori 1976, Marshall 1971, Reneau et al. 1975, Wong and Griffin 1976). Krone (1968) summarized the filtration process. Case I was that the bacteria were larger than the pores and were strained at the soil surface. When the bacterial cells accumulated on the soil surface, they became the filter. Case II, called bridging, occurred when the cells were slightly smaller than the pores. In this case the cells traveled into the soil until a pore opening was too small. Then the cell "bridged" between two soil particles, and cells behind it were filtered out. Case III, called straining and sedimentation, included cases I and II, as well as removal of small bacteria by fluid passing through the very fine pores and crevices. The adsorbing nature of a soil depended on the texture and composition of the soil, the nature of the cations around the soil, the number of bacterial cells present, pH, electrolytic concentration, and other factors.

Active bacterial movement is greatly affected by the water content of a soil. Bacteria are restricted to movement in water filled pores or water

films, and as the water content decreases more bacteria are retained in the soil. Griffin and Quail (1968) presented suggestions for defining the limitations of the physical regime permitting bacterial movement in soils. They were: Bacteria depend on a continuous water pathway where the water filled pores have a greater pore neck diameter than 2 to 3 um, and the lense of water in very large pores must be sufficiently large and be in contact with a number of soil particles. They used soil and aluminum grit columns, 1 cm deep, and varied the moisture content. Their results demonstrated that a critical volume of water was necessary for appreciable movement of bacteria to occur. Griffin and Quail concluded that movement of bacteria in most soils was very restricted if the soil was much drier than field capacity.

Hamdi (1971 and 1974) studied the active movement of Rhizobium trifolii in soils. His results generally agreed with those reported by Griffin and Quail. Wong and Griffin (1976) studied the active movement of different species of bacteria through soils. Bacillus subtilis and Azotobacter did not move appreciably through natural soils at matrix potentials greater than -150 cm of water.

There have been a few investigators that have used soil columns to determine the movement and retention of certain bacteria. Bitton et al. (1974) was interested in the differences between bacterial movement in saturated and unsaturated soils. Four soils were used, but a sandy soil was used for most of the experiments. Their results indicated that non-encapsulated bacteria were least affected by soil water content. They postulated that the encapsulated bacteria were larger and therefore more subject to filtration. The bacteria did not move with the water when the water content dropped below 15 percent in the sandy soil.

Survival of Fecal Indicator Organisms in Soil

It has been reported that the detection of fecal indicator organisms suggests the occurrence of pathogenic organisms from sewage (Wilson et al. 1968). The potential health hazard is dependent on the retention of sufficient numbers of pathogenic bacteria in water to transmit disease (McFeters et al. 1974, Geldreich and Kenner 1969). In a laboratory study this group noted the decline of fecal streptococci and fecal coliforms isolated from domestic sewage. The reduction in numbers of pure cultures of these indicator organisms followed a two-log reduction per day. This was well correlated with reduction kinetics for Salmonella typhimurium and Shigella dysenteriae (McFeters et al. 1974). By comparison the decline was much more rapid in pure cultures than under natural conditions (McFeters et al. 1974). Similar results were observed by Hyde (1976) on the survival of Streptococcus faecalis in sewage sludge. This pathogenic organism survived at least 7 months after only one application (Hyde 1976). Additional studies have been performed suggesting that disease producing bacteria decrease exponentially outside the normal host but that this decrease is related to the adversity of the environment (Mack et al. 1958). Adaption to the outside environment, however, is possible, and potential hazard is dependent on the level of contamination, the specific type of bacteria, and on conditions in the soil and water (Mack et al. 1958).

The significance of Salmonella-contaminated water is realized in the reports of Craun (1974) and Reasoner (1974). These independent researchers concluded that surface waters could serve to transmit disease to man. Their studies were performed on salmonellae shed in the feces of infected animals which contaminated water supplies.

Some question has been raised as to the validity of the presence of fecal organisms as an indication of the presence of pathogenic bacteria (Fair et al. 1967 and 1971). Benarde (1973) reported the recovery and persistence of pathogenic bacteria in the absence of detectable coliforms. He suggested that coliforms do not indicate anything but the presence of fecal matter. Another group has suggested that salmonella can become widely distributed in surface waters in the apparent absence of coliforms and could therefore pose a potential health hazard (Cherry et al. 1972). The use of fecal indicators for determining potential presence or absence of intestinal pathogens then remains questionable although at present no other rapid method exists.

Survival and Movement of Viral Particles through Soils

Most wastes are treated, or stored, before application to land, and the populations of microorganisms in the wastes are altered (Elliott and Ellis 1977). Pathogenic microorganisms are poor competitors outside the host and either die or just survive rather than proliferate in treated wastes. Viruses are more resistant than bacteria to chlorination and significant numbers remain active after chlorination (Foster and Engelbrecht 1973). Wellings at al. (1974) reported that there was a threefold reduction in viruses due to chlorination. These results are not directly applicable to the sewage treatment system used at San Angelo because chlorination has not been used to treat the wastes before land application.

More than 100 different viruses are known to be excreted in the feces of man (Benarde 1973, Rivers and Horsefall 1959). Viruses of primary concern are those regularly excreted in large quantities in the feces of infected individuals; infectious hepatitis and entero virus (Berg 1964). The only documented cases of waterborne viral outbreaks have been due to viral hepatitis (Clarke and Kabler 1964).

A factor of importance in considering waste application to land is the movement of viral particles through soils. There have been several investigators that have studied the effects of soil on viral percolation. Most agree that soil can very effectively filter viruses but some viruses do pass through most soils (Drewry and Eliassen 1968, Duboise et al. 1974, Dugan et al. 1975, and Young and Burbank 1973). McMichael and McKee (1965) failed to isolate virus from primary effluent and secondary chlorinated sewage that was percolated through 61 cm of soil. Gilbert et al. (1976) also experimented with removal of viruses from wastewaters by land application. They determined that the number of viral particles were reduced by four logs from percolating a viral solution through 9 mm columns of sandy loam soil.

Some investigations have determined factors affecting viral percolation. Drewry and Eliassen (1968) reported that the cation concentration of the liquid affected adsorption of viral particles and that soils with high clay or silt content readily adsorbed viruses. They concluded that the removal of viral particles from percolating waters was primarily due to adsorption and that viruses were not likely to be leached into ground waters. Cooper et al. (1975) and Wellings et al. (1974) published data showing that viral movement through soils was increased when distilled water was used for leaching. Both Duboise et al. (1976) and Cooper et al. (1975) have demonstrated that viruses were released from soils with addition of distilled water. The leachability of different types of viruses may vary considerably. Young and Burbank (1973) stated that polio viruses were less susceptible to adsorption onto soil surfaces than other viruses.

Little information exists on factors influencing virus persistence in soils. Gerba et al. (1975) has reported that kaolinite clay and cations may prolong virus survival in sand. Virus adsorption onto clays plays an important role in viral removal, but cannot be equated to their inactivation (Bitton 1975). Bagdasar'yan (1964) reported that enteroviruses adsorbed on loamy and sandy loam soils remained infective and Schaub et al. (1974) has shown that viruses adsorbed to clay were just as infectious as free viruses.

PARASITOLOGICAL STUDIES

Parasitic Protozoans

One of the major protozoans in sewage is Entamoeba histolytica, the causative agent of human amoebiasis which varies in its effect on humans from mild abdominal discomfort involving diarrhea alternating with constipation to chronic dysentery with mucous and blood. This parasite can be extremely dangerous if it becomes extra-intestinal. Approximately 12 percent of the people in North America are infected with this parasite (Belding 1952). High levels of occurrence of this parasite have been reported to be related to certain occupations and income levels. It is interesting to note that only 9 percent of agricultural workers cultivating irrigation fields where normal irrigation procedures were used have been shown to harbor E. histolytica, while over 14 percent of municipal sewage plant operators have been shown to be infected with this parasite (Mitchell 1972). Tsuchiya (1940) has demonstrated that 2 to 5 percent of humans are infected with E. histolytica but do not have clinical symptoms from it and act as carriers that are not generally detected.

Entamoeba histolytica has been demonstrated from sewage in many parts of the world. Several authors have noted the presence of this parasite in sewage and have examined its transmission to man from sewage (Dixon and McCabe 1964). Faust and Russell (1957), Tobie (1840), Scott and Littig (1962), and other authors found that numerous animals served as vectors to carry this parasite from sewage treatment plants to humans. Primarily flies have been implicated but cockroaches, dogs, and rodents have also served

as vectors. Pipkin (1949) concluded that in areas where human sewage is not protected and flies have easy access to it, there is a much greater chance for spread of E. histolytica. Brooke (1964) suggested that primitive sewage disposal systems such as surface or pit privies were responsible for contamination of the rural environment with this parasite.

Faust and Russell (1957) showed that human fecal contamination such as that incurred when sewage wastewater and sludge were applied to crops, was responsible for the spread of amoebic dysentery. The threat was especially large where large gatherings of people were established in close proximity to the disposal site. In sewage, cyst viability reduced by 30 percent for each 10°C rise in temperature (Cheng 1973).

Another protozoan which has been detected in sewage is Giardia lamblia the causative agent of human giardiasis. Depending on the geographic location within the United States, infection ranged from 1 to 20 percent (Healy 1969). When pathogenic, this parasite causes diarrhea, abdominal pain and loss of weight (Noble and Noble 1971). Its presence apparently interferes with fat absorption in the small intestine. Generally, this disease is more serious in children than in adults. This parasite has been implicated with sewage contamination in a few cases (Moore 1969 and Scott and Littig 1962). But Scott and Littig also indicated that animals, such as the fly, could transport this parasite as well.

There are a number of other parasitic protozoans which potentially could be found in sewage and sludge. Two of these are Entamoeba coli, an amoeba which in most cases is not pathogenic in man, and Naegleria gruberi, a pathogenic amoeba which causes fatal amoebic meningocephalitis in man (Duma et al. 1969).

Parasitic Helminths

There are numerous parasitic worms which are of concern to public health. Fortunately, several of the more pathogenic species (eg. Clonorchis sinensis, human schistosmoes, and Paragonimus westermanii) have not been introduced into North America apparently because of the lack of susceptible intermediate hosts.

In the United States, Taenia saginata, the beef tapeworm of man, causes clinical symptoms such as abdominal pain, digestive disorder, loss of weight, and a variety of other symptoms (Belding 1952). Adult tapeworms in the intestine of man discharge a million or more eggs per day in the feces of infected humans. Cattle may become infected by grazing on pastures where sewage wastewater has been used for irrigation or by drinking grossly polluted water (Mitchell 1972). The intermediate stage of this parasite normally develops in the muscle of cattle but man has been shown to be an occasional accidental host for this stage as well.

Because of this parasite large numbers of cattle are condemned each year by USDA inspectors. Man becomes infected with the adult tapeworm by eating poorly cooked beef that contains the intermediate stage of this parasite.

The eggs of T. saginata have frequently been observed in sewage (Hamlin 1946). The eggs were able to survive for 335 days under cool moist conditions (Silverman and Griffith 1955). Cheng (1973) states that the eggs of this species were commonly transported by birds.

Ascaris lumbricoides is an intestinal nematode in man as an adult but will also make a larval migration through the blood system and lungs before reaching the intestine (Belding 1952). Adult females may discharge 200,000 or more eggs per day which can remain viable in water or soil for months. This parasite has been found in about 20 percent of the sewage operators and 16 percent of the farmworkers where sewage irrigation is used (Mitchell 1972). Monitoring of sewage in Poland is used as an index of the incidence of ascariasis in the human population (Iwanczuk and Stobnicka 1968). Raw sewage from Darmstadt, Germany, has been shown to contain 540 eggs of this parasite/100 ml of fluid (Mitchell 1972). The high density of this parasite in Germany was attributed to application of raw sewage to gardens. Similarly, viable eggs of A. lumbricoides have been detected in the Colorado River downstream from where a chlorinated sewage effluent was discharged (Wang and Dunlop 1954). Some livestock, especially pigs, could become infected by grazing on sewage irrigated fields and act as a reservoir for this parasite.

Other ascarids, such as Toxacara sp. which normally are parasites of dogs, were shown on the national news last year to cause a serious disease in children called larval migraines. This can be very serious especially when the larvae invade the brain and central nervous system of the host. This genus may be in municipal sewage if pet feces are disposed of in household toilets, but pet feces can also reach the sewage treatment facility in gutter washings.

Hookworms, Necator americanus and Ancylostoma duodenale, are debilitating parasites of humans. Symptons are loss of energy and anemia and resemble malnutrition. This disease is endemic in the southeastern United States. Humans are infected by a filariform larvae which penetrates through the skin. In addition, hookworm infections are known to occur in higher than normal levels where sewage is used for irrigation. In his book, "The Plague Killers", Greer Williams sums up the efforts of years of attempting to eliminate human hookworms by pointing out the eradication is not possible and that proper disposal of waste products from human and reservoir hosts and that wearing of shoes are the only means by which this group of parasites can be kept in check.

Other parasites (e.g. Diphyllobothrium latum, Dipylidium caninum, Echinoccus sp.) are human parasites which could possibly be associated with sewage wastewater and sludge.

Currently Fox and Fitzgerald (1976) from the University of Illinois have been monitoring the kinds of parasites in sludge and have found A. lumbricoides, Toxacara sp., Trichuris sp., Teania sp. and strongyloid type nematode eggs, as well as some coccidia. Fitzgerald and Ashley (1976) have been investigating the survival of the egg of Ascaris sp. in sludge.

Parasite Life Cycles

The following are life cycle diagrams of the more prevalent parasites found in this study. Figures 1, 2, and 3 are life cycle of Entamoeba histolytica, Strongyloides sp., and Eimeria, respectively.

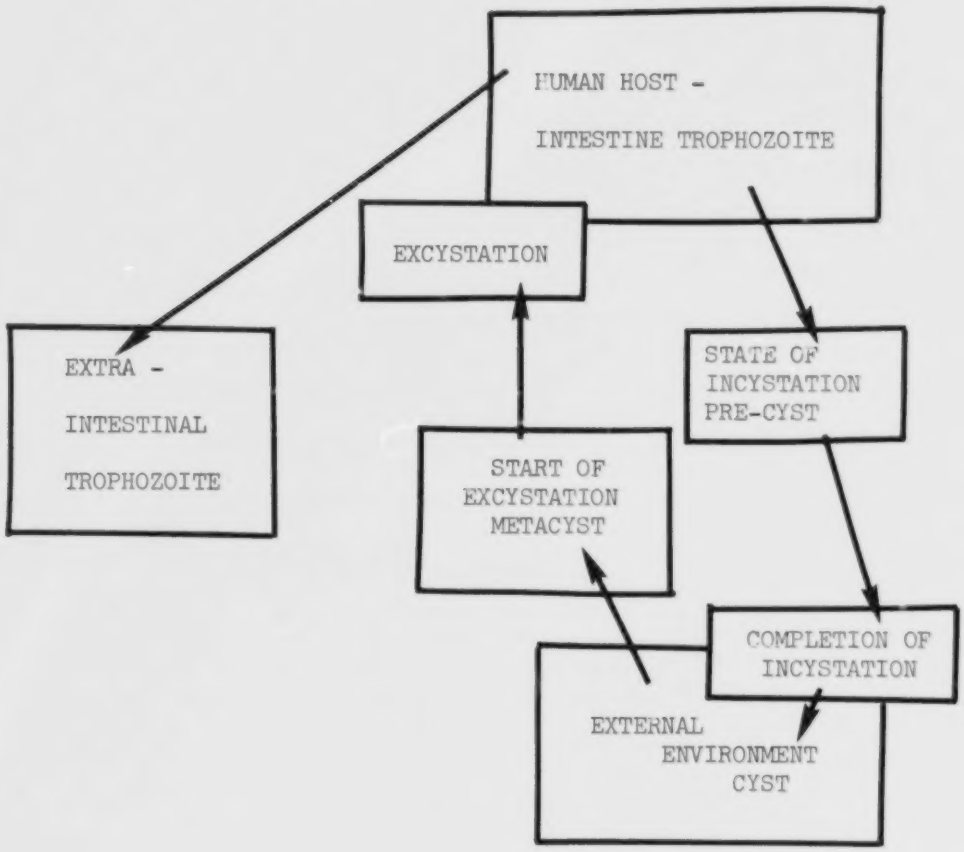

Figure 1. The life cycle of Entamoeba histolytica.

Human infected by ingestion of cyst stage from polluted water, infected food handlers, flies contaminating food, night soil cultivation and direct introduction of cyst. The same life cycle has been demonstrated for E. coli.

Giardia sp. has a very similar life cycle to that described above for E. histolytica. Extra-intestinal implications are known only for E. histolytica.

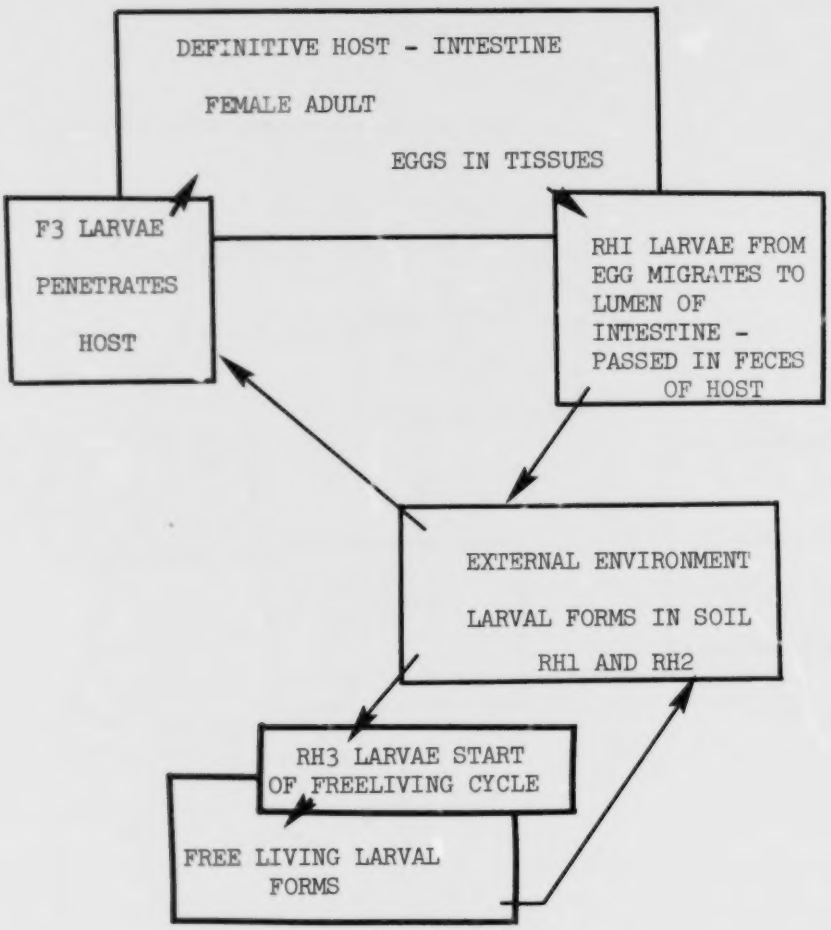

Figure 2. The life cycle of Strongyloides sp.

Infection of definitive host by F3 larvae either from environment or by internal or external autoinfection.

Haemoncus contortus has a similar life cycle to that found in the parasitic portion of Strongyloides sp. except that eggs rather than larvae are passed in the feces and hatch in the environment.

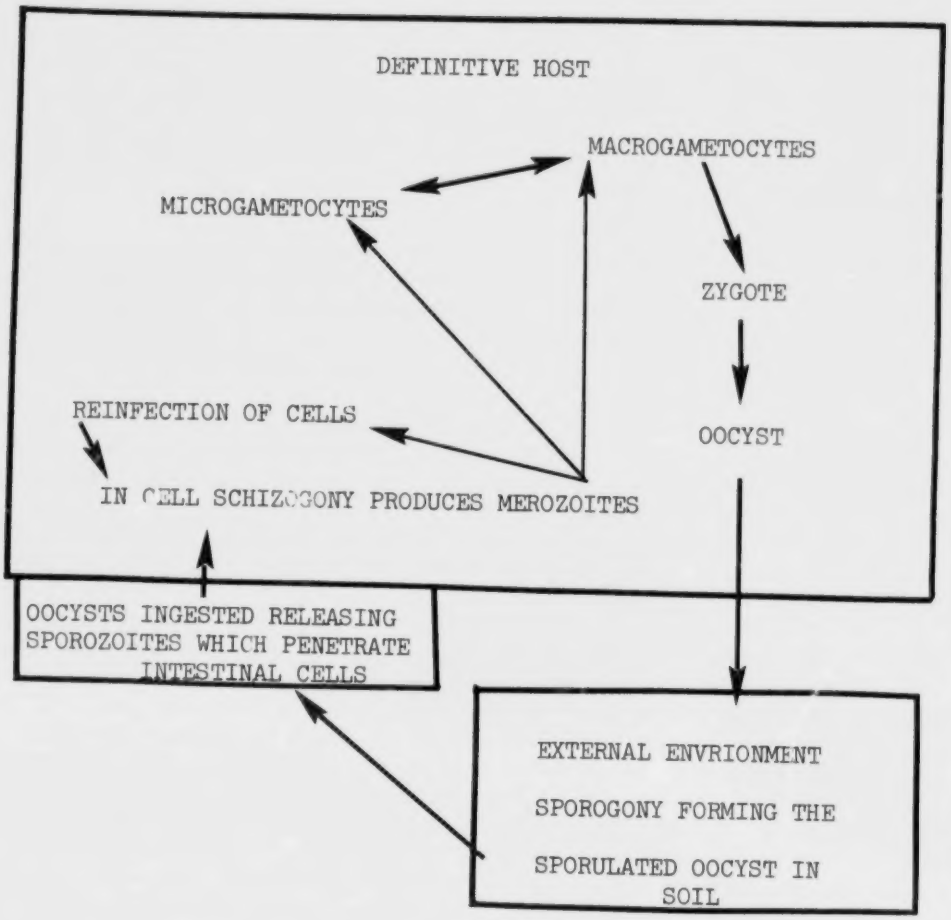

Figure 3. The life cycle of Eimeria sp.

Infection of the definitive host is by ingestion of the sporulated oocyst.

SECTION 5

MATERIALS AND METHODS

SITE DESCRIPTION

The main study area was the sewage farm operated by the City of San Angelo, Texas. This city has a population of approximately 67,000 inhabitants. The sewage disposed of on the sewage farm was predominantly from households with less than seven percent of it contributed from industries. The average daily flow of sewage to the farm was approximately 0.24 m^3/sec.

Treatment of the sewage was minimal before application to the land. During the time of the study, a new sewage treatment facility was under construction and much of the old facility was inoperable. The incoming sewage was put through a primary filter and distributed to the sewage lagoons. Water for irrigation was pumped from the lagoons. Retention time in the lagoons was not known because there was no set schedule for using the lagoon water for irrigation.

The farm consisted of 259 ha of land used for pasture and hay production and 77 ha of cultivated land. Approximately 600 cattle were normally being grazed on this land. Generally, small calves were not kept on the farm. All the land on the farm was highly productive because neither water nor fertilizer nutrients were limiting.

The fields of the sewage farm were levied to prevent surface runoff of water. Irrigation was accomplished by flooding. Internal drainage of the soils was very good, and much more water was applied than was needed to meet the requirements of the crops. The excess water drained through the soils and surfaced in seepage creeks on lower areas of the farm. The seepage creeks drained into the Concho River.

MICROBIOLOGICAL STUDIES

Field Study on Bacteria

Soil and Water Sampling

Water, sewage, and soil samples were collected for bacteriological analysis at monthly intervals. Samples were evaluated as to total aerobic bacterial counts, bacterial indicators of human pollution, and potential human pathogens. These included total and fecal coliforms, *Salmonella* sp., *Shigella* sp., enterococci, and *Pseudomanas aeruginosa*.

A total of eleven water and sewage samples were collected per month. These included one sample of raw sewage taken at the entrance to the sewage plant; the second, third, fourth, and fifth samples were taken from each of the four sewage lagoons; the sixth sample was taken from a deep well used for watering livestock; the seventh, eighth, and ninth samples were taken from each of three seepage creeks entering the Concho River; the tenth sample was taken from the Concho River approximately 6 miles upstream from the sewage facility; and the eleventh sample was taken from the Concho River approximately 6 miles downstream from the sewage facility. In addition, irrigation water was collected when irrigation of the fenced plots occurred.

Samples were aseptically collected in sterile milk dilution bottles. Raw sewage, sewage from each lagoon and seepage creek, as well as river water samples, were collected at a depth of 15-30 cm. The well sample was collected from the delivery pipe after flushing with approximately 37 liters of water.

A total of 17 soil samples were collected on each sampling. These included a sample from the fenced control plot and samples from each fenced irrigation area. Cattle were grazed on all areas except the fenced control plot. In addition, an off-farm control sample was taken.

Soil cores (2.5 cm diameter), 10 cm deep, were collected and transferred to sterile, airtight plastic sample bags. All samples were refrigerated on ice until processing.

Sample Processing

Samples were returned to the laboratory at Texas A&M University and processed within 36-48 hours after collection. Ten ml of each water or sewage sample and 10 g of each soil sample were transferred to 90 ml sterile NaCl (0.85%) blanks and diluted serially to effect a log dilution range from 10^{-1} through 10^{-8}. Ten grams of soil from each sample were dried and weighed to determine water content so that microbial counts could be reported as organisms per gram dry weight soil.

Total aerobic bacterial counts were determined by the pour plate method. One ml from each sample dilution was added to 1% nutrient glucose agar (Difco) and incubated for 48 hours at 37°C. After incubation the colonies were counted.

Total coliform counts were made by making serial dilutions and spread plating onto Eosin Methylene Blue (EMB) or Endo Agar (Difco). Incubation was for 24 hours at 37°C. All colonies exhibiting a typical green sheen were counted as presumptive coliforms. Ten percent of the presumptive coliform colonies were transferred to Difco Triple Sugar Iron Agar (TSIA). Cultures demonstrating acid/acid and no hydrogen sulfide reactions were transferred into lactose broth for final confirmation. Fecal coliform counts were obtained by making serial dilutions on EMB or Endo Agar. Plates were

incubated at 44.5°C for 24 hours. All colonies demonstrating a green metallic sheen were counted as presumptive fecal coliforms. Confirmation consisted of picking colonies to TSIA and lactose broth as per the total coliform confirmation. This was followed by determining the IMViC reactions of those typical coliforms. Only those organisms yielding an IMViC of ++--, +---, and -+-- and grown at 44.5°C were reported as fecal coliforms.

The presence of fecal streptococci was determined by making serial dilutions and spread plating onto m-Enterococcus Agar. Plates were incubated overnight at 37° C. Presence of the organism was confirmed by hydrolysis of esculin on Bile Esculin Azide Agar (Difco) and by making catalase tests.

The population of Pseudomonas aeruginosa was determined by spread plating of serial dilutions onto Pseudosel Agar (Biquest Laboratories). Colonies producing diffusible blue-green pigments were counted, and confirmation was by the cytochrome-oxidase test.

Salmonella Isolation from Soil and Water

Samples were taken of both raw sewage and irrigation effluent for isolation of Salmonella sp. Samples were collected and maintained at ambient temperature for approximately 48 hours before processing, but after processing the remainder of the samples were stored at approximately 10° C. Ten, twenty, and thirty ml samples were enriched in tetrathionate broth (TT), tetrathionate broth + 1% lactose (TTL), tetrathionate broth + 1% lactose and 1% glucose (TTLG), tetrathionate broth at pH 4.5, and tetrathionate broth at pH 5.0. Enrichments were incubated at 37° C for 18 hours after which time log dilutions were made (10^{-1} to 10^{-4}) and plated onto Xylose Lysine Desoxycholate (XLD) agar. Plates were incubated 24 to 48 hours at 37° C and suspect salmonella colonies picked to Triple Sugar Iron Agar (TSIA). Organisms yielding typical salmonella reactions on TSIA (AK++ or AK-+) were subjected to further biochemical and serological analysis.

Twenty-one and 45 days after sampling 10 to 30 ml aliquots of both samples were re-examined for the presence of salmonella by tetrathionate broth enrichment followed by plating onto XLD agar. Biochemical and serological analyses were performed as above on presumptive salmonella isolates.

Two sample locations, sites 13 and 6, were chosen for collection of soils for the isolation of Salmonella sp. Two enrichment media, tetrathionate (TT) and tetrathionate broth + 1% lactose (TTL), and two selective plating media, Bismuth sulfite (BS) agar and Xylose Lysine Desoxycholate (XLD) agar were used for isolations. Thirty and 50 g of each of the soils were placed into each enrichment, 1 part soil to 10 parts enrichment. After incubation at 37° C for approximately 18 hours log dilutions ranging from 10^{-2} to 10^{-6} were made and plated onto each of the media. After incubation at 37° C for 24 to 48 hours, suspect colonies from each dilution were picked to Triple Sugar Iron Agar (TSIA). Those TSIA which gave typical salmonella reactions were treated to biochemical analysis.

Concentration and enrichment techniques were used to isolate Salmonella sp. from the Concho River and seepage creeks. The enrichment media were tetrathionate broth (TT), tetrathionate broth + 1% lactose (TTL) and nutrient broth with 1% lactose (NL).

River and seepage creek waters were filtered through .45 µm filters by negative pressure and placed into the various enrichments according to Table 1. The volume filtered was limited by the quantities of suspended solids.

TABLE 1. VOLUMES OF LIQUID FILTERED AND MEDIA USED FOR DETERMINATION OF SALMONELLA SP. IN WATER FROM THE CONCHO RIVER AND IN WATER FROM SEEPAGE CREEKS

Sample No. and Site	Volume Filtered	Medium
No. 7 (Seepage Creek No. 3)	4L	TT
	2L	NL
	2L	TTL
No. 8 (Seepage Creek No. 2)	3L	TT
	2L	NL
	2L	TTL
No. 19 (Upstream Concho)	500 ml	TT
	250 ml	NL
	250 ml	TTL
No. 20 (Downstream Concho)	500 ml	TT
	300 ml	NL
	250 ml	TTL
No. 11 (Seepage Creek No. 1)	400 ml	TT
	200 ml	NL
	200 ml	TTL

All preparation of media, filtration, and enrichment were performed at San Angelo to prevent loss of potential salmonella due to prolonged storage. Enrichment media was kept refrigerated during transportation back to the laboratory at College Station where they were incubated at 37°C for approximately 18 hours. After incubation, log dilutions (10^{-2} to 10^{-4}) were plated onto BS agar and XLD agar and again incubated at 37°C for 24 to 48 hours. At the time of plating of the enrichment, a serial transfer of 10 ml of primary enrichment was inoculated into freshly prepared secondary enrichments of the same medium. Again after 18 hours of incubation at 37°C log dilutions were plated onto BS and XLD agar. Suspect colonies from each dilution of all plates were picked to TSIA and

biochemical analysis performed where indicated. No Salmonella sp. were confirmed from any of the enrichments.

Due to the lack of success with prior enrichment techniques, samples for Salmonella sp. were collected and enriched according to the method of Moore (1971) and Moore et al. (1969). The procedures consisted of two highly absorbent commercial tampons (Kotex) tied to a cotton cord. These were suspended in the raw sewage inlet, primary settling tank, sewage lagoons, seepage creeks, and up and downstream Concho River. The cord was anchored with weights and equipped with a conventional plastic float to maintain the tampons in the flowing water or sewage. These were allowed to remain in place for 3 to 5 days. At the end of this period, the tampons were enriched in 200 ml of freshly prepared TT broth and incubated at 45° C for 48 hours. Dilutions of the enrichment were prepared and plated onto XLD and BS agar. Colonies typical for Salmonella sp. were picked and characterized biochemically.

Salmonella Isolation from Bovine Manure

To determine whether or not cattle could become potential reservoirs of salmonellae after being pastured on the sewage farm, a study was initiated. Ten cows, not previously exposed to the farm environment, were chosen for the study. The cattle were transferred to farm pastures after a manure sample was collected. Six samplings were made over a 7 month period. When a sufficient quantity of manure was available, 50 g was enriched in 200 ml of TT broth for 18 hours, dilutions were made and plated onto BS and XLD agars. At the time of initial plating, serial transfers of 10 ml were made to freshly prepared TT broth and processed as previously described. Suspect colonies were examined biochemically for typical salmonella reactions.

Bacterial Analysis of Well Water Samples

Wells were sampled both on and off the sewage farm. The water samples were examined for total aerobic bacteria, and fecal and total coliforms. The procedure for enumeration and confirmation was as previously described for water samples.

Bacterial Analysis of Soil Core Samples

Core samples from two soils present on the San Angelo sewage farm were enumerated for coliform bacteria. The soil series used were Rio Concho and San Angelo. Six cores from each soil were taken; three from inside the sewage farm and three from outside the sewage farm. The cores were sectioned and the bacteria in each section were enumerated. The sections were from the soil depths: 0-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50 50-100, and 100-150 cm. Soil from each depth was mixed thoroughly, and 10 g of soil from each section was added to 95 ml of saline. This was ground in a Waring blender for 1 minute. A dilution sequence from the soil slurry was conducted and was plated on m-endo agar (Difco). Pour plates were used on the soil slurry from the blender. Spread plates were used on higher dilutions. Plates were incubated at 37° C for 24 hours. Colonies exhibiting a characteristic green sheen were considered coliform bacteria.

Laboratory Studies with Bacteria

Soils

Soils chosen for this investigation represent important agronomic and economic soils of Texas. Soil types were: Arenosa loamy sand, San Angelo sandy clay loam, Houston Black clay, and Beaumont clay. The San Angelo soil was collected from the San Angelo sewage farm, where sewage had been applied for the last 15 years. The soil samples were collected from the A horizon, air-dried, and ground to pass through a 2 mm mesh sieve.

The soils had a wide variety of physical characteristics (Tables 2 and 3). The pH was determined according to the procedure of Davis (1943). The soil texture was determined by the Bouyoucos hydrometer method (Day 1965). Organic matter was measured by using a colorimetric variation of the Walkley-Black method (1934). Hydraulic conductivity (cm/hr) and permeability (cm^2) of the soils were determined by the procedure of Klute (1965). Electrical conductivity was determined on a 2:1 liquid-soil suspension using a conductivity bridge. Soil moisture characteristics were measured by applying pressure to soils on porous plates (Neilson 1958 and Richards 1949). Total porosity was determined by measurement of the bulk density using the paraffin clod method (Russell 1949). By using soil moisture characteristic curves (Figure 4), pore-size distributions were derived by the method of Vomocil (1965).

TABLE 2. PHYSICAL AND CHEMICAL CHARACTERISTICS OF FOUR SOILS USED IN LABORATORY STUDIES ON LEACHING OF BACTERIA

Property	Arenosa loamy sand	San Angelo sandy clay loam	Houston Black clay	Beaumont clay
Sand	84%	47%	11%	20%
Silt	6%	18%	35%	30%
Clay	10%	35%	54%	50%
pH	4.9	7.3	7.5	5.5
Organic matter	1.3%	3.6%	3.1%	2.4%
Hydraulic conductivity	5.81 cm/hr	0.61	0.51	0.16
Electrical conductivity	230 µmhos/cm	680	480	220
Pore space	53%	58%	52%	46%

TABLE 3. PORE SIZE DISTRIBUTION OF THE SOILS USED IN LABORATORY STUDIES ON LEACHING OF BACTERIA

Pore-size (μm)	Arenosa loamy sand	San Angelo sandy clay loam	Houston Black clay	Beaumont clay
30,000 - 150	11%	15%	12%	4%
150 - 75	7%	10%	13%	10%
75 - 50	18%	10%	7%	7%
50 - 22	31%	5%	8%	14%
22 - 12	4%	15%	15%	27%
12 - 9	1%	1%	1%	1%
9 - 6	1%	1%	1%	1%
6 - 3	0%	1%	1%	1%
3 - .6	1%	3%	3%	7%
.6 - .3	0%	4%	4%	6%
Total	74%	65%	65%	78%

Inoculum Preparation

Four strains of fecal related bacteria were used in this experiment. Laboratory strains of Escherichia coli 11303 and Salmonella typhimurium were obtained from Dr. B. G. Foster, Biology Dept., Texas A&M University. Strains of Salmonella enteriditis and Streptococcus fecalis were isolated from the San Angelo, Texas sewage farm.

Biochemical tests were conducted for confirmation of the bacterial species. The salmonella strains were streaked on brilliant green agar (Difco) for growth. Tests were conducted using Triple Sugar Iron agar (Difco), Lysine Iron agar (Difco), urea agar (Difco), Simmon's citrate agar (Difco), tryptophane for indole production, MR-VP medium (Difco), dulcitol, arabinose, mannitol and malonate. The isolates were then tested for agglutination by Salmonella O Antiserum (Difco), and typed. The strain of E. coli was streaked on MacConkey's agar (Difco) for growth. The same biochemical tests were then conducted as with the salmonella. Streptococcus fecalis was streaked on m-enterococcus agar (Difco), and for confirmation as a group D streptococcus bile esculin azide agar (Difco) was used. Additional biochemical tests to determine the species were grown on mannitol, arabinose, saccharose, and glycerol hemolysis, and gelatin liquification. All biochemical reactions were incubated at 37°C for 24 hours.

The E. coli and the strains of salmonella were grown in a 50 ml Erlenmeyer flask containing 20 ml of nutrient broth. An initial inoculum was added, and the organisms were incubated at 37°C for 10 to 14 hours on a rotary shaker, until a cell concentration of 10^9 organisms per ml was

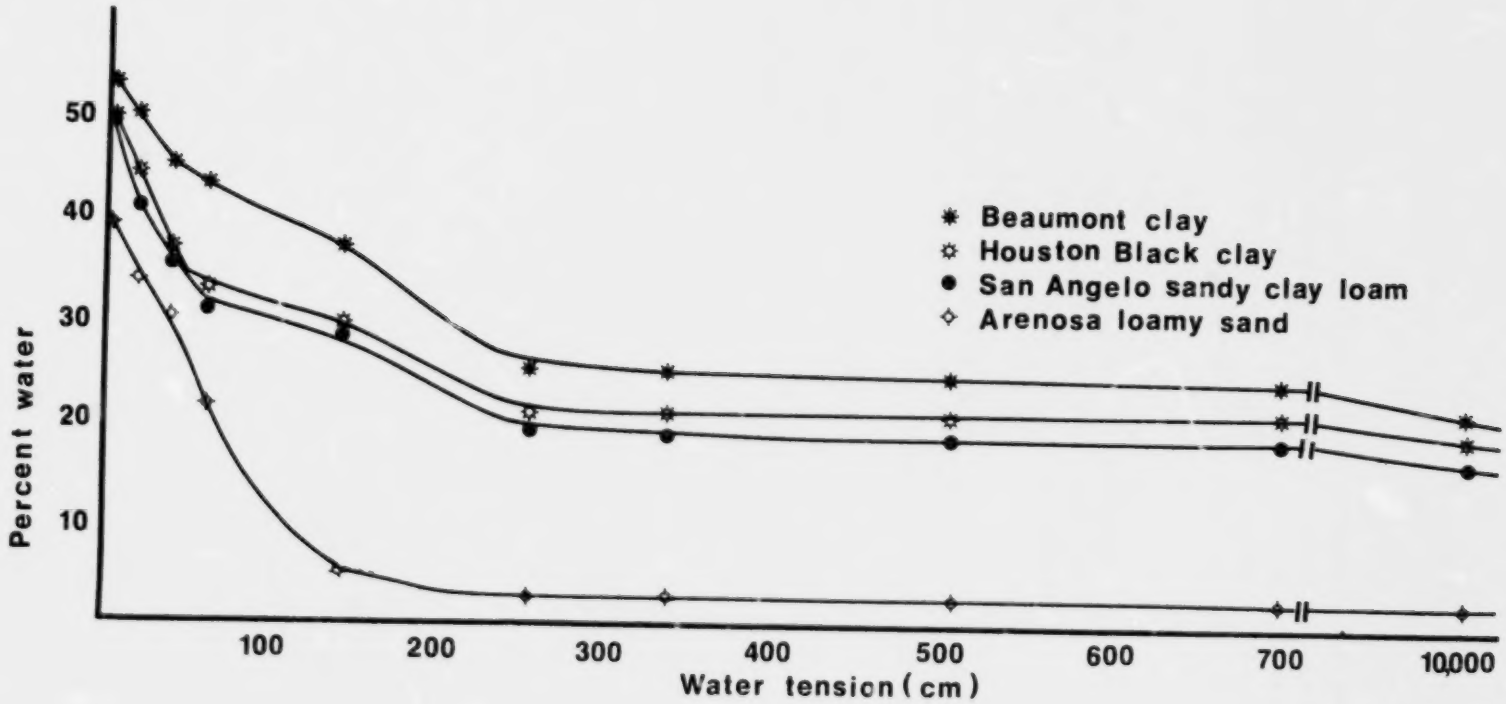

Figure 4. Water holding capacity of the four soils used in laboratory studies on leaching of bacteria.

reached. Streptococcus fecalis was grown in tryptic soy broth (Difco). These bacteria only obtained a cell concentration of about 3 x 10^8 organisms per ml.

Dilutions of bacteria were performed using physiological saline (0.85 percent NaCl in distilled water) to establish a total bacterial concentration of 10^6 organisms per ml. Five ml of the bacterial suspension was then added for each cm of soil in the columns. The amount of bacterial suspension added to each column is shown in Table 4.

Column Preparation

Columns were developed that would retain soil, pass water and bacteria, and that could obtain a -1/3 atmospheric tension on the soil with applied suction. The columns consisted of a modified millipore swinnex (25 mm) filtering assembly, with a 22 mm diameter pyrex tube inserted into the top portion of the assembly. The bottom section was attached to a small glass tube which passed through a rubber stopper. This entire column assembly could then be attached to a 500 ml filtering flask. Figure 5 shows a representative column assembly.

A 3 um nucleopore filter was chosen as the best supporting grid to pass the liquid and bacteria. Filters were tested for optimal removal of gravitational water, but that would also pass bacteria. Filters tested were: 3, 5, and 8 um nucleopore, 8 um cellulose filters (Selectron), and 5 and 10 um teflon filters (Millipore).

Soil column heights of 1, 2, 3, 4, 5, 10 and 15 cm were used. The representative soils were added to the columns in 5 g increments and compacted to a reproducible bulk density. Compaction was by means of a #1 rubber stopper attached to a glass rod dropped 10 times from a height of 15 cm. This procedure was repeated until the desired column height was obtained.

The inoculum was carefully poured onto the soil surface so that the soil surface would not be disturbed. The water was allowed to percolate through the soil until the wetting front visually reached the bottom of the column, then a vacuum of 0.8 to 0.9 atmospheres was applied until visible drainage from the column had stopped. Bacteria in the leachate and in the soil were then enumerated.

Adsorption to Soil

The capacity of bacteria to adsorb to particles greater that 1 um in diameter was estimated by the following procedure. Twenty-five g of soil and 25 ml of a liquid suspension containing 10^6 bacteria per ml were mixed in a 250 ml centrifuge tube. This mixture stood for 5 minutes before an additional 220 ml of saline was added. The soil was suspended by shaking on a mechanical shaker for 5 minutes, and centrifuged (IEC #2 head no. 226, 1500 rpm, 3:30 minutes) to remove any particles greater than 1 um (Day 1965). The supernatant was decanted, mixed, and the bacteria were enumerated. An additional 220 ml of saline was added to the sediment in the

TABLE 4. INITIAL INOCULUM OF BACTERIAL SUSPENSION, PER ML, ADDED TO EACH COLUMN[a]

Soil column height	Milliliters of inoculum added
1 cm	5
2 cm	10
3 cm	15
4 cm	20
5 cm	25
10 cm	50
15 cm	75

[a] -10^6 org/ml

tube and the procedure was repeated. Salmonella typhimurium and Escherichia coli were the bacteria used in this experiment.

Effect of Salts on Leaching

All previous experiments in this study were conducted using physiological saline as the transport medium. Therefore, additional tests were conducted using other salts in solution at varying concentrations. These tests were conducted to determine the effects of different salts on the leaching characteristics of bacteria through the soils. One salt mixture contained an equal number of sodium and calcium ions consisting of 2/3 CaCl and 1/3 NaCl. Three concentrations were used: 0.31 N, 0.155 N, and 0.077 N. These concentrations represented 1, 1/2, and 1/4, respectively, the normality of physiological saline. The other salt solution was 0.01 M phosphate buffer (K_2HPO_4) in distilled water. These salt solutions were inoculated with S. typhimurium and passed through 5 cm soil columns. The bacteria in the leachate were then enumerated.

Distribution in Columns

A study was conducted to determine the numbers of bacteria present in different sections of a soil column after an inoculum of bacteria had passed through the soil. Fifteen cm columns, inoculated with S. typhimurium, were sectioned and the bacteria present in each increment of soil were enumerated. Soil sections examined were 0-3, 3-5, 5-10, and 10-15 cm.

Saturation of Soils

Salmonella present in the leachate were determined after 4 additions of Salmonella typhimurium were made to soil columns. Four 25 ml additions of salmonella were added to 5 cm soil columns of Arenosa sand and San Angelo soil. Four 5 ml additions of salmonella were added to 1 cm columns of Houston Black clay. Columns were allowed to drain before the next addition of bacteria was made.

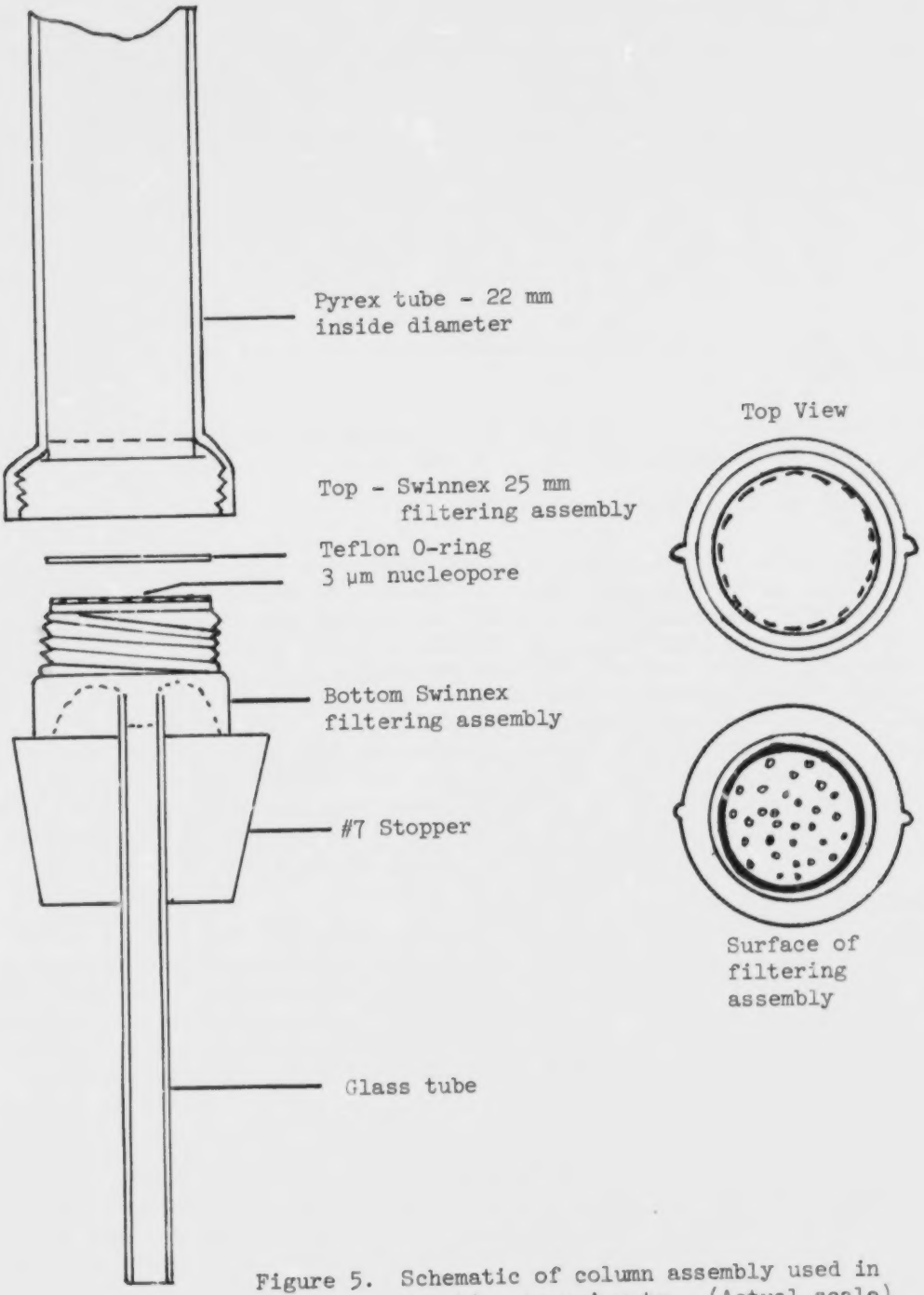

Figure 5. Schematic of column assembly used in leaching experiments. (Actual scale)

Breakthrough Characteristics

Breakthrough characteristics were conducted with 5 cm soil columns to determine the movement of the bacteria in relation to the movement of the water. Twenty-five mls of bacterial suspension in saline were added to the soil surface. Bacteria in each 2 ml increment of the leachate were enumerated.

Enumeration

Bacteria in the leachate that passed through the soil columns were enumerated by making serial, ten-fold dilutions (Table 4). A 1 ml Eppendorf pipette and 9 ml sterile saline blanks were used for the dilution sequences. A 0.1 ml Eppendorf pipette was used to deliver the suspensions onto the desired plating medium. The spread plate method was used for spreading the inoculum on the plates.

Bacteria from the initial dilution that was used to inoculate the soils were enumerated. From this, the total number of bacteria applied to the soils could be determined so that all tests could be standardized by comparing a fixed constant to the initial concentration of bacteria. Initial concentrations of bacteria added to the soil are presented in Table 5.

The plating media used for this investigation consisted of brilliant green agar (Difco) for enumeration of salmonella, m-endo agar (Difco) for E. coli, and m-enterococcus agar (Difco) for enumeration of S. fecalis. The media were prepared 1 day in advance and allowed to dry at 37°C overnight. Colonies exhibiting characteristic reactions with the respective medium were counted.

Size

Preparations of the bacteria were photographed using phase/contrast light microscopy to determine the respective size distribution of the bacteria. The bacteria were grown in their respective medium, and a drop was placed on a microscope slide. Two percent agar (liquid) was added to the suspension and covered by a coverglass.

Statistical Analyses

To determine significant differences between bacterial numbers present in the leachate from the column experiments, statistical analyses were performed on the differences between the proportions of bacteria leaching through the soil. The proportions of bacteria were determined by comparing the amount of bacteria present in the leachate to the total amount of bacteria that leached through the soil at a particular depth. The standard deviation of bacteria at each depth was used to determine significance between the amount of bacteria that leached through the soil at a particular depth. Differences were determined by Duncan's multiple range test using the 95 percent confidence level. Differences in numbers of bacteria in the salt experiments were determined by the Student's t-test.

TABLE 5. CONCENTRATION OF INOCULUM ADDED TO EACH SOIL

Depth	Soils		
	Arenosa loamy sand	San Angelo sandy clay loam	Houston Black clay
	million/ml		
1 cm			
E. coli	5.8	1.0	1.0
S. fecalis	1.1	1.6	0.5
S. typhimurium	0.9	0.9	1.8
S. enteriditis	1.6	1.4	3.0
2 cm			
E. coli	2.0	1.5	1.2
S. fecalis	1.1	1.6	0.5
S. typhimurium	1.7	0.9	1.8
S. enteriditis	1.6	3.6	3.5
3 cm			
E. coli	8.0	2.0	1.0
S. fecalis	2.7	2.0	0.5
S. typhimurium	1.7	0.9	1.8
S. enteriditis	1.6	3.6	3.5
4 cm			
E. coli	8.0	1.2	1.0
S. fecalis	2.7	2.0	0.5
S. typhimurium	0.6	0.9	1.8
S. enteriditis	1.6	3.6	2.9
5 cm			
E. coli	1.1	9.1	1.0
S. fecalis	1.7	1.7	0.5
S. typhimurium	0.6	9.0	1.0
S. enteriditis	1.4	1.7	1.6
10 cm			
E. coli	1.1	8.8	1.0
S. fecalis	2.0	2.0	0.5
S. typhimurium	2.2	0.9	1.0
S. enteriditis	3.0	1.6	2.1
15 cm			
E. coli	1.1	1.1	1.0
S. fecalis	2.6	2.6	0.5
S. typhimurium	2.2	4.3	2.6
S. enteriditis	3.0	1.6	2.1

Field Study on Viruses

Soil and Water Sampling

Water and soil samples were collected at the San Angelo site at monthly intervals. These samples were collected at the same sites and times as the bacteriological samples, primarily from the seepage creeks and the sewage lagoons. Sample sizes normally consisted of 10 to 15 ml. Each water sample was frozen (-70°C) and thawed prior to filtration and inoculation.

Each soil sample was ground separately in a mortar and then mixed in 5 ml of Hank's balanced salt solution. After thorough mixing the soil particles were allowed to settle. The supernatant fluids were decanted into a centrifuge tube and centrifuged (1000 RPM) for 10 min. This fluid was decanted and stored at -70°C.

Tissue Culture

The protocol for the tissue culture system was developed using Buffalo monkey kidney cells (BGMK) or African Green monkey kidney cells in culture. The growth medium selected consisted of Eagle minimum essential medium with Earle's salts supplemented with fetal bovine serum, L-glutamine, and tryptose phosphate broth. The maintenance medium was identical to the growth medium except the serum level was reduced from 10 percent final concentration to 1 percent.

Enumeration

A preliminary filtration study was conducted to determine the effect of serial filtration of viral samples to eliminate bacterial contaminants. Both 0.45 um and 0.22 um millipore filters were used in this study. Cultures of Group 3 Reovirus exhibiting 4+ CPE on BGMK were first frozen and then thawed before filtration. The outgrowth of the cultured cells to confluency was accomplished in 48-72 hours. Separate aliquots of each virus dilution were inoculated onto the BGMK cells. The test was read 6 days after inoculation.

The reduction of viruses, due to serial filtration, prompted the filtration technique for samples to be altered to a single passage of the liquid through a 0.22 um membrane. The filtrate was inoculated directly onto substrate tissue in replicates of 3-5 tubes per sample. An inoculum of 0.25 ml of filtrate was used throughout. All cultures were read after 6 days. Those samples exhibiting CPE in culture were either passaged or re-inoculated onto other BGMK cultures.

Laboratory Studies with Viruses

Soils

The two soil types used in these experiments were a San Angelo fine sandy loam and Houston Black clay. Their characteristics are given in Tables 2 and 3.

Column Preparation

Column assemblies were identical to those used in the bacteriological column studies with the same supporting grid (3 μm nucleopore). Soil column heights of 1, 2, 3, 4, 6, 10, and 15 cm were used. The representative soils were added to the columns in 5 g increments and compacted to a reproducible bulk density.

Inoculation and Enumeration

Columns were separately charged with 4 ml sample (1 ml of Reovirus, TCID 50 1 x 10 4.09/ml in 3 ml Hank's BSS). Five ml of water or saline solution (0.85% NaCl) were added to the column surface to provide eluant for each 5 ml fraction collected. Eight to twelve fractions were collected per column. The eluate fractions were stored at -60°C until assayed in tissue culture.

Group 3 Reovirus was used because of its stability between pH 2.2 and 8.0 and its survival abilities. Assays were conducted on BGMK tissue in culture. Mono layers were grown in Leighton tubes in Eagle minimum essential medium containing 10 percent v/v fetal bovine serum, 200 units of penicillin G and units of streptomycin per ml. Each column leachate fraction was filtered through a 0.22 μm millipore filter. Tissue cultures, inoculated with 0.25 ml filtrate, were incubated at 37°C for 6 days and monitored for virus associated cytopathic effects (CPE).

Distribution in Columns

Soils from each column were divided into thirds to represent the top, center and bottom of each soil column. These were retained in bulk and frozen (-60°C) until assayed for residual virus in tissue culture.

For assay the soil was thawed and mixed with 5 ml of Hanks BSS and shaken by hand. After the soil settled, the supernatant fluid was decanted, filtered through a 0.45 μm HA millipore membrane, and 4 replicates of Leighton tubes were inoculated with 0.25 ml of filtrate. These were monitored for any viral associated cytopathic effects (CPE).

PARASITOLOGICAL STUDIES

Detection of Possible Human Parasites in Sewage

Sewage and Water Sampling

Fluid samples were taken from the incoming raw sewage, the primary settling tank, storage lagoons and the irrigation effluent and examined for possible human parasites. A 500 ml sample was taken monthly from each location in an attempt to quantitate the number of parasites in the sewage. Samples were placed in sterilized 500 ml glass bottles and transported to the laboratory on ice for examination. All samples were refrigerated at 5°C until examined. Most samples were examined within 48 hours after collection.

Sample Processing and Examination

Samples were thoroughly mixed and 25, 50, 75 and sometimes 100 ml subsamples were concentrated using 47 mm diameter millipore filters having 5 µm pores. The concentrate was removed from the filter with the edge of a 22 x 22 cm glass coverslip and resuspended in 1 ml of Lugol's iodine solution. Subsamples of over 100 ml were normally too cluttered with sediments to be adequately examined microscopically. Suspensions were uniformly mixed with a pipet to prevent clumping and 6 to 10 wet mounts were made to determine if protozoan cysts, helminth eggs or larvae of possible human parasites were present. If none were observed the subsample was discarded and the next larger volume subsample was concentrated and examined. If parasites were observed in wet mounts, portions of the concentrated sample were examined using the white blood cell counting grids of a hemocytometer. Usually, 3 to 5 hemocytometer observations were made on each suspension using the 8 white blood cell grids. In some preparations, some of the 8 grids could not be examined because of excess debris. The volume per white blood cell counting grid was 1 mm x 1 mm x 0.1 mm = 0.1 mm^3. The references used in identification of protozoan cysts and helminth eggs and larvae were primarily "Basic Clinical Parasitology" (Brown 1969); "Textbook of Clinical Parasitology" (Belding 1952) and "Laboratory Guide to Medical Protozoology and Helminthology" (National Naval Medical Center, Bethesda, Maryland). Unfortunately, nuclei could not be distinguished in 50 to 70 percent of the suspected protozoan cysts; thus, size and general appearance were used in classification. Size and general appearance may not be a good indicator for the diagnosis of human protozoan parasites.

Detection of Possible Human Parasites in Sludge

Sampling

During the last half of this project it became apparent that examination of sludge for possible human parasites would be important. Six to ten core samples were taken each month from a sludge lagoon that had been allowed to go dry in September of 1975. Sampling of the sludge material started in January of 1976 and was continued through August of 1976. The depth of sludge in this lagoon was between 15 to 25 cm. Core samples were placed in 500 ml widemouth glass jars and transported on ice to the laboratory for examination; samples were refrigerated in the laboratory at 5°C until used.

Sample Processing and Examination

One-gram subsamples were taken from the surface of core samples and approximately 15 cm below the surface for comparison. Examination of these subsamples were by direct iodine wet mounts and zinc-sulfate flotation (Cable 1958). In the zinc-sulfate technique, each of two 1-gram subsamples were suspended in 10 ml of lukewarm water in a centrifuge tube. These suspensions were centrifuged for approximately 1 minute at 2600 rpm and the supernatant fluid discarded. This process was repeated until the supernatant was clear. After the final rinse the tube was filled to capacity with a zinc-sulfate solution (specific gravity = 1.180) and the sediments

resuspended. Centrifuge tubes were covered with a glass coverslip to pick up floating materials. The coverslip just touched the surface of the liquid. The remaining material was centrifuged at 1800 rpm for 1 minute. After centrifugation coverslips were again floated on the liquid in the tubes. This time they were allowed to float for 10 to 15 minutes and then placed on a glass microscope slide with a little iodine stain for microscopic inspection.

Detection of Nematode Larvae in Soil

Sampling

Soils were sampled to determine if possible human and/or livestock parasites could be detected. Sampling was initiated in October 1975 and was terminated in January 1976 in favor of a controlled study monitoring parasite levels in cattle. On-farm parasite levels were compared t the levels observed on an adjacent control site where sewage had not been used for irrigation. Detection of nematode larvae was primarily by the Baerman method (Cable 1958).

Samples were taken from a 30 cm square area from which all vegetation but that within 2 cm of the soil surface was removed. Samples included remaining grass, forbes plant debris, and the top 2m of soil. We attempted to take at least 10 on-farm samples and 4 off-farm samples each month. Sampling was occasionally disrupted by excessive irrigation or rain on fields of the sewage farm. Field samples were transported on ice in plastic zip-lock bags to the laboratory where they were stored at 5°C until examined.

Sample Processing and Examination

Samples were thoroughly mixed (vegetation and soil) and 50 ml packed subsamples were placed on two layers of gauze over a wire screen mounted in a large glass funnel. Funnels were freshly filled each time with 40°C water so that the water level in the funnel just touched the bottom of the wire screens. The stems of funnels were fitted with surgical tubing which was clamped off to hold the water in the funnel. The funnels with subsamples were allowed to stand undisturbed for at least 1 hour. Nematode larvae were attracted towards the warm water and accumulated in the stem of the funnel. The larvae were harvested in a centrifuge tube after releasing 25 ml portions of water from rubber tube into centrifuge tubes. Most of the larvae were in the first 25 ml but additional 25 ml portions were drained into centrifuge tubes until no more larvae were present. Further concentration was possible by centrifugation of these 25 ml samples at 1800 rpm. Nematode larvae harvested were counted in a gridded watch glass with a 5X wide dissecting microscope fitted with 20X ocular lenses. When necessary, for identification, larvae were examined with a compound microscope.

Detection of Parasites in Livestock Feces

Sampling of Livestock on the Sewage Farm

A study was established to compare the parasite levels of sheep and cattle on the sewage farm to those grazing on a nearby farm. Sampling was initiated in September 1975 and terminated in January 1976. Samples were taken from freshly deposited feces and placed in 500 ml glass containers and transported on ice to the laboratory for examination. Samples were stored at 5° C in the laboratory until examined.

Sample Processing and Examination

On thawing, samples were thoroughly mixed and subsamples used for analyses. Examination of these samples was by direct iodine wet mounts by the Baermann funnel technique and by zink-sulfate flotation as previously described in this report under the sections on sludge and soil processing and examination.

Monitoring of Parasite Buildup in Cattle Feces

Cattle

Ten cattle were assayed for parasite levels before being released on the sewage farm. The average weight of the cattle was approximately 400 pounds. Each animal was treated with 8 mg/kg of Levasole, marked with ear tags and released on the farm for observation. Animals 1 through 5 were retreated with the drug after 2 months to see if an antihelminthic drug would hold parasite levels down. Three cattle already on the farm were randomly selected to serve as controls to determine how quickly parasite levels would increase to those already present.

Sampling

Monthly fecal samples were removed directly from all ten cattle, placed in 500 ml sterilized glass containers and transported to the laboratory for examination. Samples were stored at 5° C until used.

Examination

The feces were examined for parasites by direct iodine wet mount examination, zinc-sulfate flotation and Stoll's egg counting technique (Cable 1958). Counting eggs by the Stoll technique was accomplished as follows: A Stoll flask was filled to the 56 ml mark with N/10 sodium hydroxide, feces was added until the level in the flask reached the 60 ml mark, ten glass beads were added and the flask was stoppered and mixed by hand shaking. From this suspension 0.15 ml was transferred to a microscope slide using a Stoll's pipette and covered with a 22 x 40 mm glass coverslip. Eggs were counted using a compound microscope. The number of eggs was multiplied by 100 to obtain the number of eggs per gram of feces. This gave only an estimate of the number of eggs present. We assumed that more eggs would indicate more parasites.

SECTION 6

RESULTS AND DISCUSSION

MICROBIOLOGICAL STUDIES

Field Study on Bacteria

Populations in Sewage, Seepage Creeks, Lagoons and the Concho River

Application of municipal sewage effluent to agricultural lands may have an affect on the microbiological quality of ground water. The population of microorganisms in the effluent has a bearing on the impact of the effluent upon ground waters. In these investigations sewage effluent was monitored monthly from the point raw sewage entered the treatment facility until some of the water reappeared as seepage water draining into the Concho River. Populations of total aerobic bacteria, fecal coliforms, enterococci, and Pseudomonas aeruginosa were determined to evaluate the bacteriological impact of the effluent on the ground water quality.

The sewage lagoons effectively reduced the populations of all groups of bacteria by approximately 90 percent. Unfortunately, the retention time in the lagoons could not be determined because there was no set schedule for piping sewage into the lagoons or taking effluent from them for irrigation.

The total aerobic counts from the different sample areas are presented in Figure 6. The raw sewage usually contained the highest numbers of total aerobic bacteria, ranging from 5×10^6 to over 10^{10} bacteria per ml. The four sewage lagoons were very similar to one another in regards to total bacteria ranging between 1×10^5 to 10^7 organisms per ml. The bacterial load present in the seepage creeks demonstrated considerable fluctuation, from about 1×10^1 to 10^4 organisms per ml. The total aerobic load of the Concho River was relatively constant, with few differences between the upstream and downstream counts. The two noticeable exceptions were a sharp increase in numbers in the downstream samples during the month of June, and the decrease in numbers in the downstream samples during October. The downstream population fluctuations in June were similar to that in the seepage creeks.

The total coliform counts from the different sample areas are presented in Fig. 7. The raw sewage contained the greatest numbers of organisms, ranging from 2×10^5 to 5×10^6 organisms per ml. The numbers of coliform organisms present in lagoons 1 through 3 were about the same, ranging from 10^4 to 10^5. However, the coliform numbers were considerably reduced in

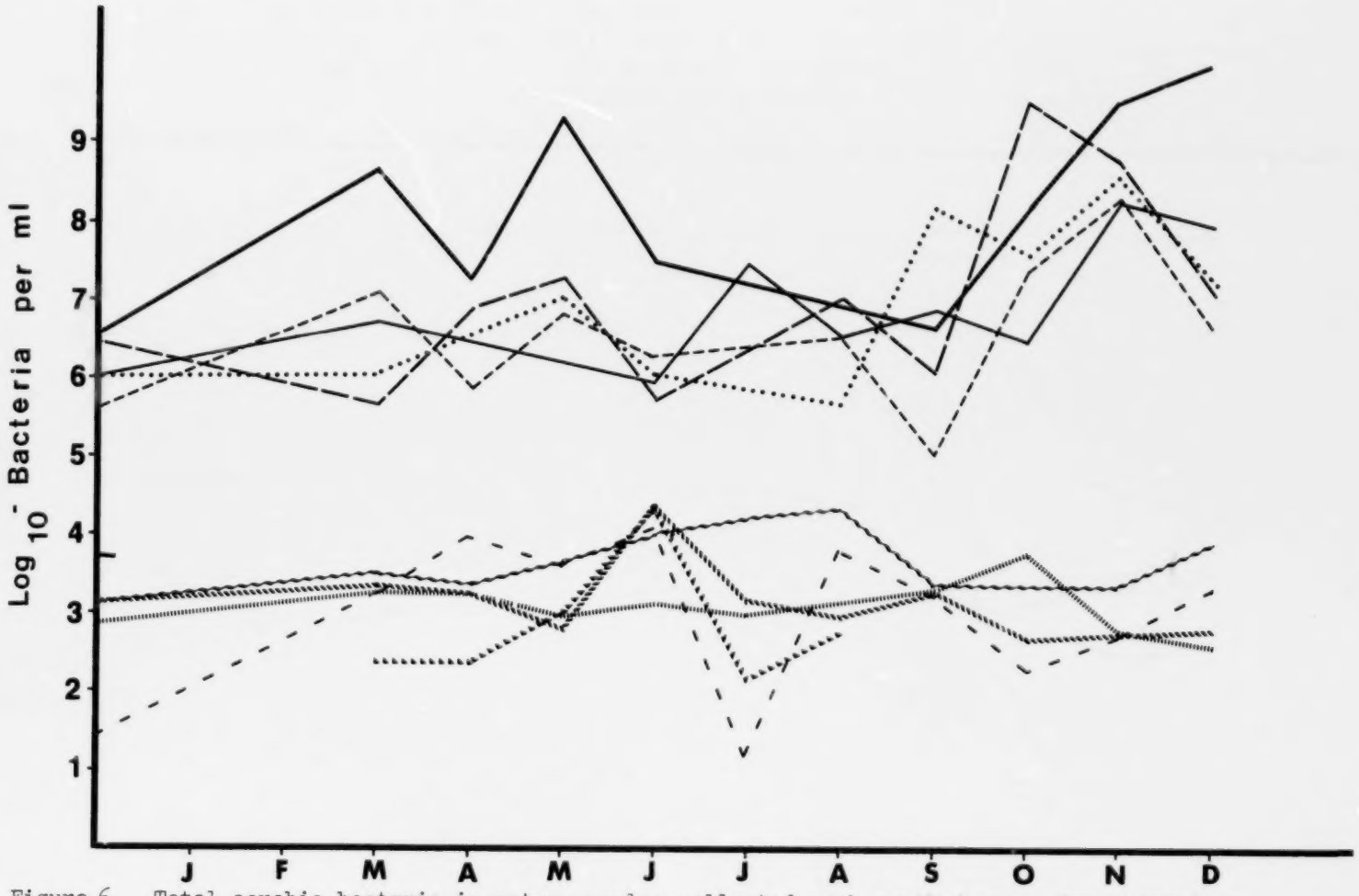

Figure 6. Total aerobic bacteria in water samples collected each month from various locations on the San Angelo sewage farm. The lines represent raw sewage ———, lagoons 1 ——, 2 — —, 3 – – –, 4 ·····, seepage creeks 1 ~~~, 2 – · –, 3 ·····, and upstream Concho River ·····, and downstream Concho River ~~~.

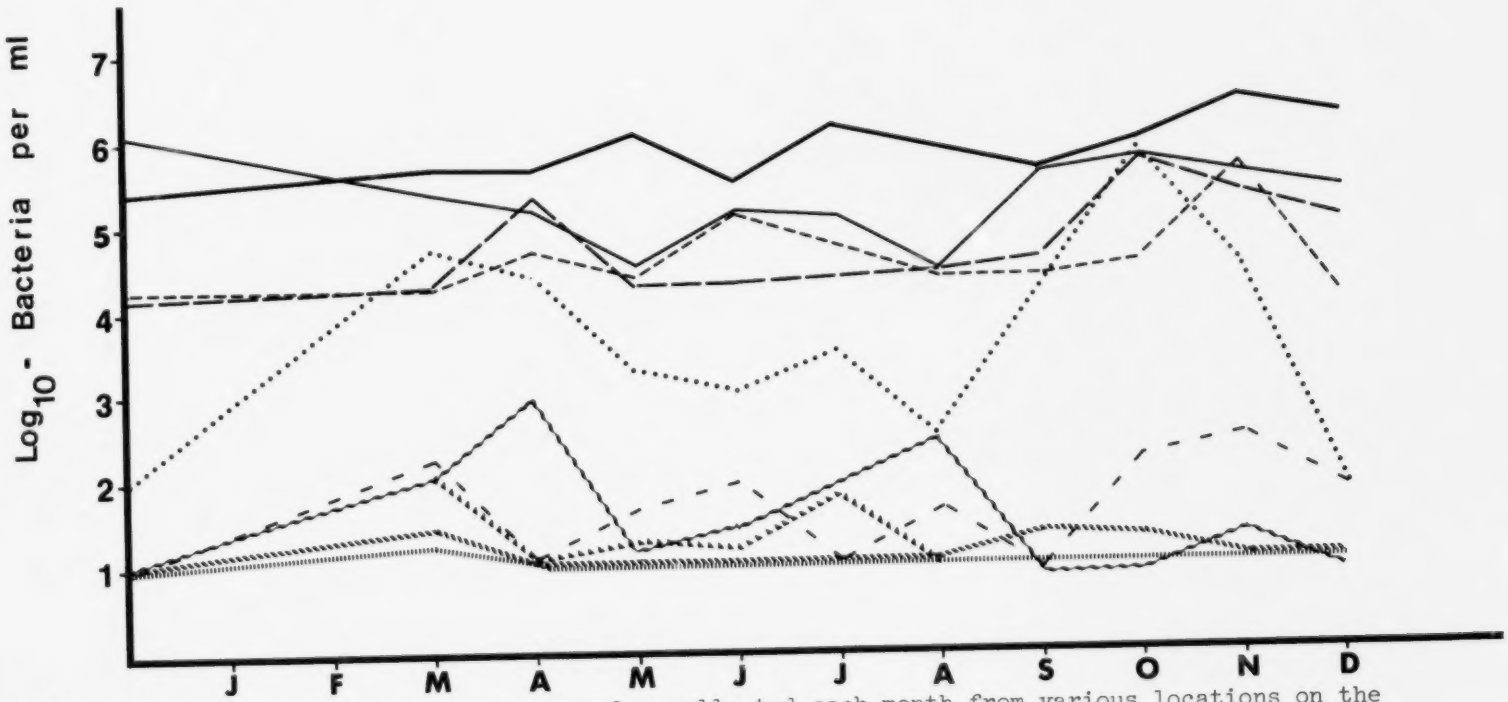

Figure 7. Total coliforms in water samples collected each month from various locations on the San Angelo sewage farm. The lines represent raw sewage ———, lagoons 1 ——, 2 — —, 3 - - - -, and 4 ·····, seepage creeks 1 — · — ·, 2 - - , 3 ·····, and upstream Concho River ····· , and downstream Concho River ·····.

4 compared to the other lagoons. The effluent had to pass through lagoons 2 and 3 to reach 4. The coliform counts in the seepage creeks fluctuated between 10^1 and 10^3 organisms per ml, but no trends could be established between these counts and the coliform count in the raw sewage. There was a slight increase in bacteria for the month of September and October for the downstream Concho samples.

The fecal coliform counts from the selected areas revealed a considerable amount of fluctuation (Fig. 8). The fecal coliform population present in the raw sewage varied between 10^3 to 10^6 organisms per ml. Fecal coliform numbers in lagoons 1 through 3 coincided very well with the fluctuations in the raw sewage, but lagoon 4 was very low in fecal coliform bacteria. The fecal coliform population in lagoon 4 was usually an order lower than the other lagoons. The population of fecal coliforms in the seepage creeks were generally much lower than in the lagoons.

The populations of Ps. aeruginosa in monthly samples varied considerably (Fig. 9). There was a sharp increase in Ps. aeruginosa for the month of April, and again in June for most samples. An increase in bacterial numbers in the lagoons was also observed in December. A greater number of Ps. aeruginosa were present in the downstream samples for April, as compared to the upstream samples. However, in some cases the upstream samples contained greater numbers of these bacteria than did the downstream samples.

The enterococci populations from the different samples are presented in Fig. 10. Again the raw sewage contained the greatest number of recoverable bacteria. A sharp decrease was observed for numbers of enterococci in the raw sewage for the month of September. However, the populations of these bacteria in the sewage lagoons did not decrease noticeably. Seepage Creek 2 contained a substantial number of enterococci compared to Seepage Creek 1. Even so, Seepage Creek 2 only contained 6×10^1 enterococci per ml. Enterococci were not isolated from the Concho River or Seepage Creek 3.

Populations in Well Water

Because the microorganisms from sewage were present in the seepage waters we examined water samples collected from 15 wells in the surrounding area to determine if sewage organisms were reaching the wells. The total aerobic counts in the wells were extremely low, ranging from less than 1×10^1 to 3×10^2 organisms per ml. No total or fecal coliforms were detected in any of the wells. Thus the sewage farm did not affect the deep ground water used for drinking.

Populations in Soil

Soil samples representing 17 collection sites were collected monthly to determine impact of sewage effluent on the population of sewage microorganisms (Fig. 11). Because there was no significant difference (5% level of significance) in populations between months, the data were averaged for brevity (Table 6). A control sample was collected from both on the sewage farm (site 20) and off it (site 60). The control area on the farm was fenced

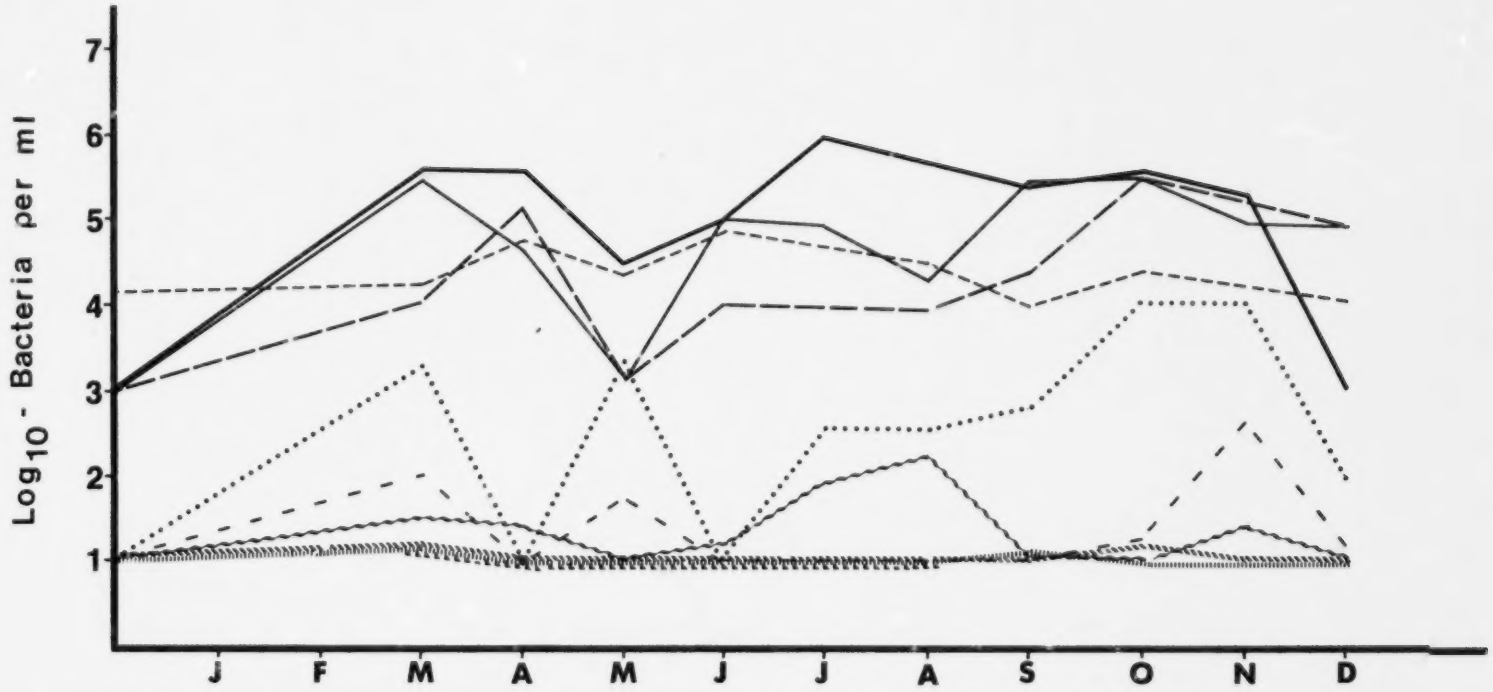

Figure 8. Fecal coliforms in water samples collected each month from various locations on the San Angelo sewage farm. The lines represent raw sewage ——, lagoons 1 ——, 2 — —, 3 ----, and 4 ·····, seepage creeks 1 ~~~, 2 - -, 3 ·····, and upstream Concho River ~~~, and downstream Concho River ~~~.

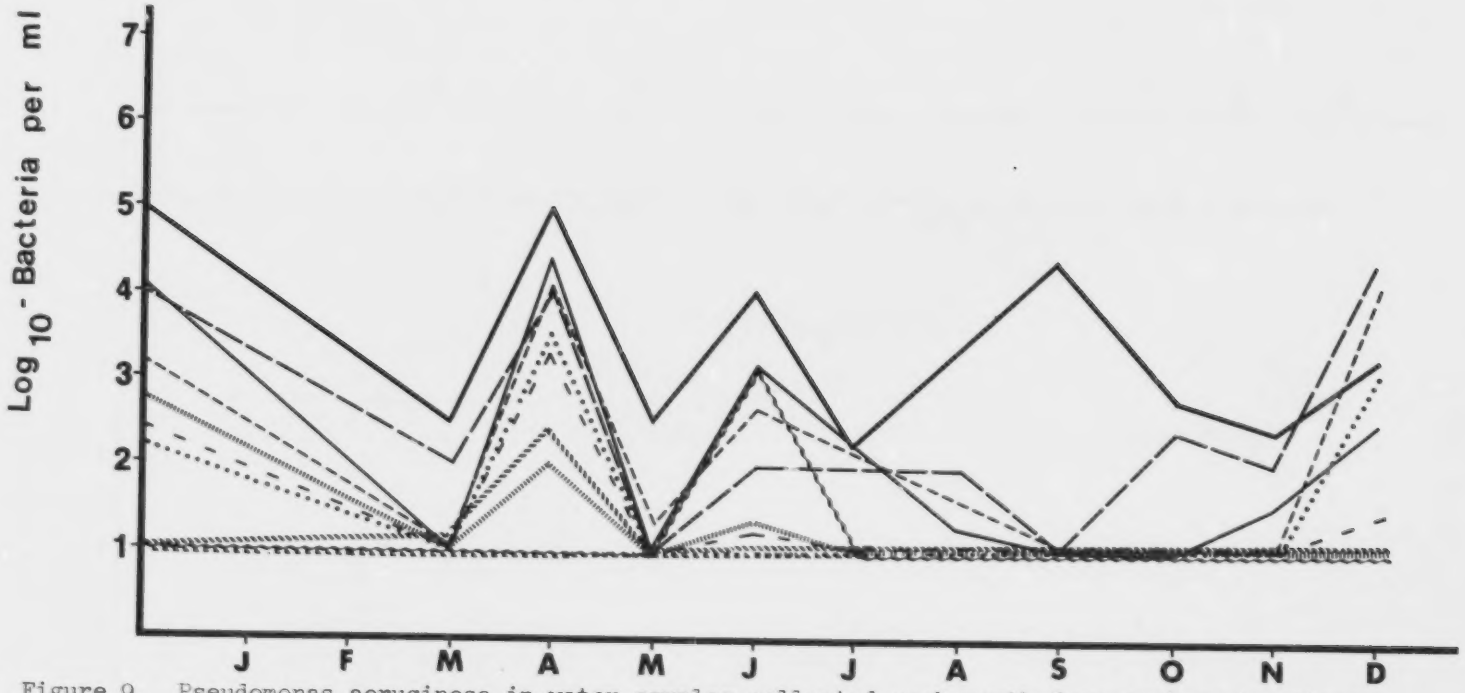

Figure 9. Pseudomonas aeruginosa in water samples collected each month from various locations on the San Angelo sewage farm. The lines represent raw sewage ——— , lagoons 1 ——— , 2 — — , 3 - - - - , and 4 . . . . . , seepage creeks 1 — — , 2 - - - , 3 . . . . . , and upstream Concho River ——— , and downstream Concho River . . . . . .

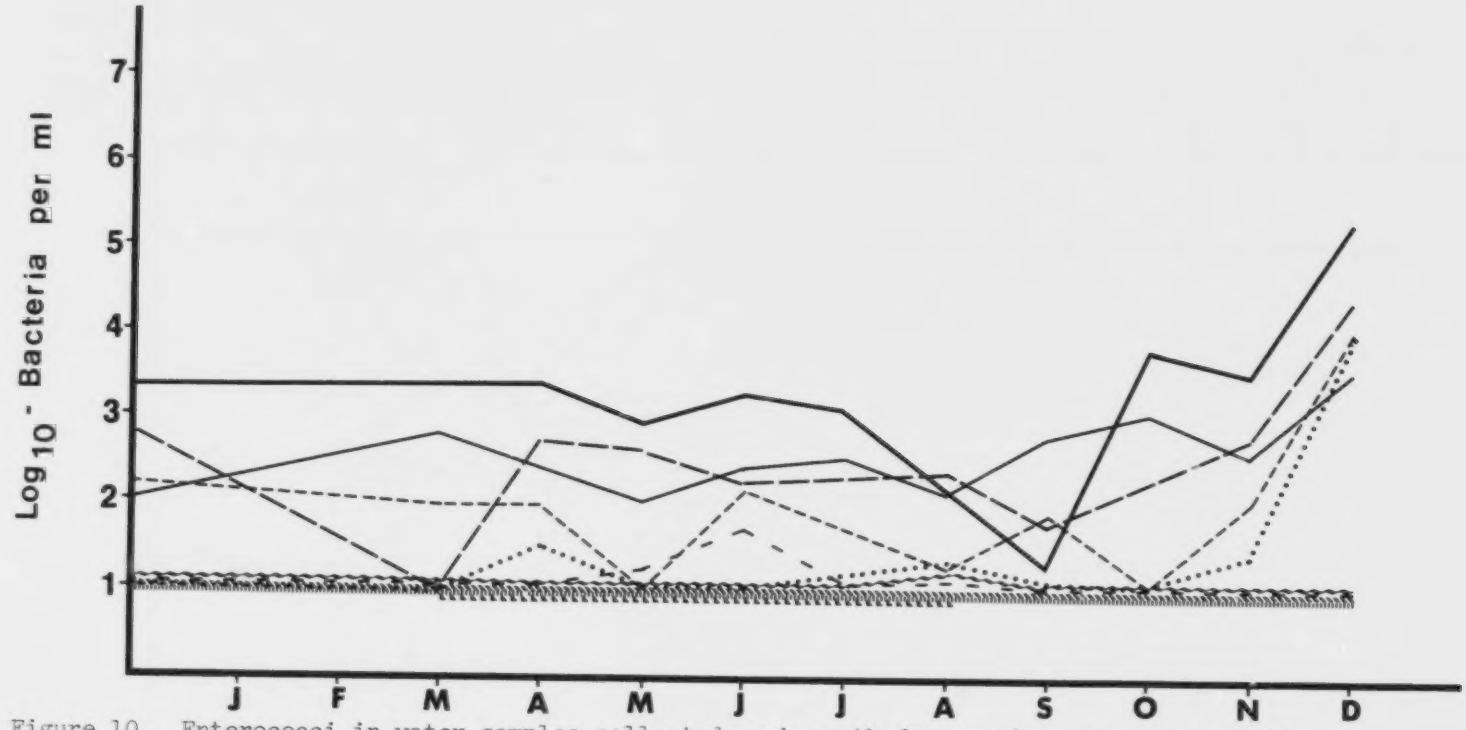

Figure 10. Enterococci in water samples collected each month from various locations on the San Angelo sewage farm. The lines represent raw sewage ——, lagoons 1——, 2 ——, 3----, and 4 ·····, seepage creeks 1 ~~~~, 2 - -, 3·····, and upstream Concho River ——— , and downstream Concho River ~~~~~.

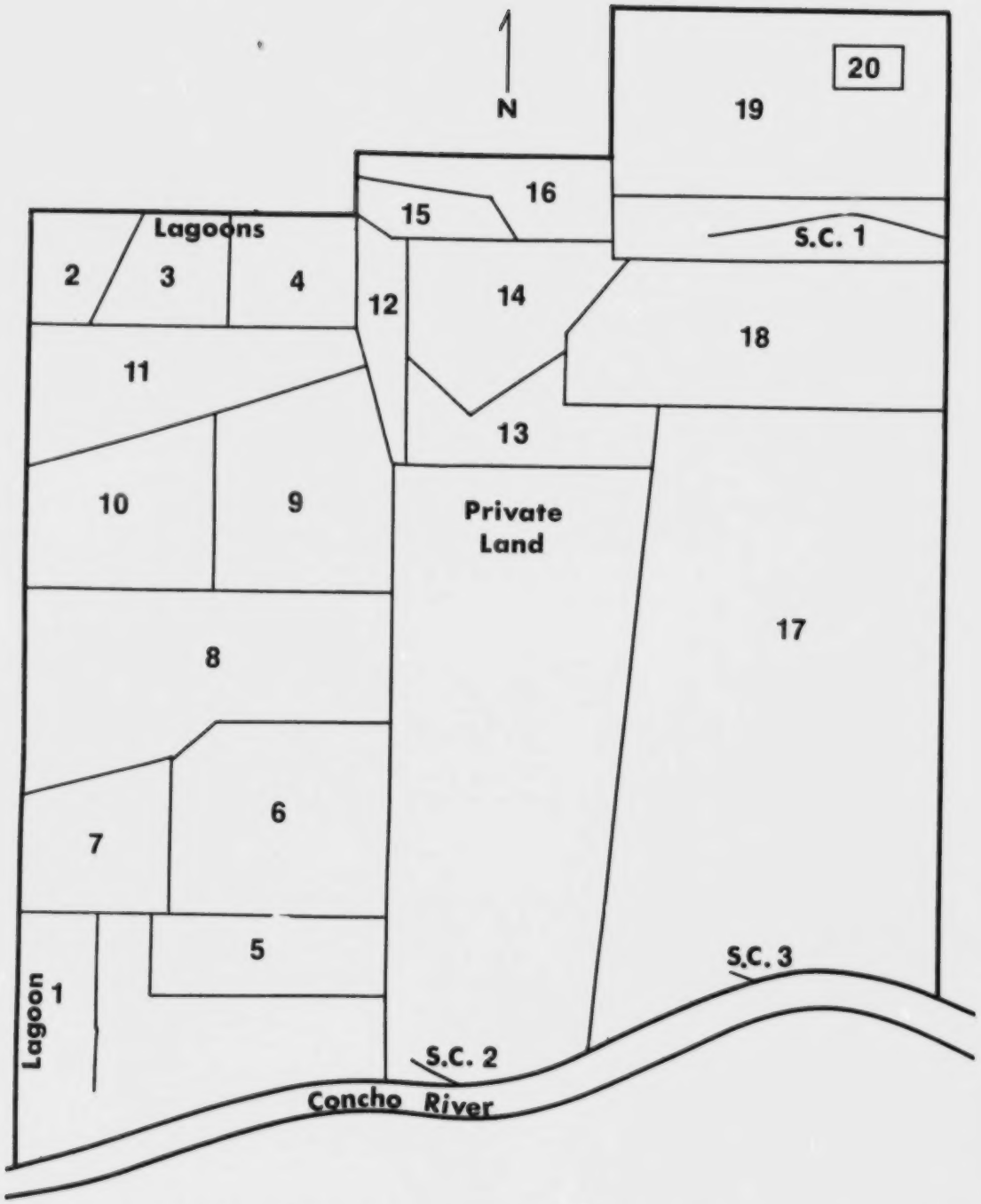

Figure 11. Location of fields, lagoons and seepage creeks on the San Angelo sewage farm.

TABLE 6. POPULATIONS OF BACTERIA IN SURFACE SOIL SAMPLES COLLECTED FROM SEVERAL SITES ON THE SEWAGE FARM AND A SITE OFF THE SEWAGE FARM

Site	Total Aerobic	Total Coliform	Fecal Coliform	Pseudomonas aeruginosa	Enterococci
			No./g soil		
20	3×10^8	1×10^3	1×10^4(3)	1×10^3	1×10^3(2)
17	6×10^7	3×10^3	1×10^3(3)	8×10^2(1)	1×10^2(3)
13	3×10^8	7×10^3(1)[a]	3×10^3(3)	9×10^2(1)	3×10^3(1)
9	5×10^7	4×10^3(1)	2×10^4(2)	8×10^2(2)	4×10^2(1)
6	4×10^8	4×10^4	7×10^4(3)	(4)	7×10^4(3)
10	4×10^8	3×10^3(1)	3×10^3(1)	9×10^2	1×10^3
11	2×10^7	3×10^3	2×10^3(1)	3×10^3(2)	1×10^2(2)
12	2×10^8	2×10^3(1)	1×10^4(1)	5×10^2(1)	1×10^2(3)
15	4×10^8	5×10^3(1)	1×10^2(3)	5×10^3(1)	8×10^2(2)
16	2×10^7	1×10^2	8×10^2(1)	4×10^3	4×10^2(2)
19	3×10^8	9×10^3(1)	8×10^2(2)	1×10^3(2)	7×10^2(2)
18	1×10^7	1×10^3(1)	3×10^3(3)	1×10^3	2×10^2(1)
8	5×10^7	2×10^4(1)	1×10^3(2)	9×10^2	1×10^3(3)
7	6×10^8	1×10^4(1)	(4)	5×10^2(2)	1×10^2(1)
5	1×10^8	3×10^4(1)	(4)	7×10^3(1)	2×10^2(1)
14	4×10^8	1×10^4	1×10^4(1)	4×10^3	1×10^3(1)
Off Farm	1×10^8	(4)	(4)	(4)	(4)

[a]The number in parenthesis is the number of samples, out of the four collected, that contained fewer than 100 organisms per g. The number of organisms indicated is the average of the samples that contained more than 100 per g.

to keep cattle out, but was irrigated with effluent. The population of the total aerobic bacteria was not different between on the sewage farm and off it (5% level of significance) and was in the range between 3×10^7 to 4×10^8 per g of soil. However, the distribution of selected types of bacteria recovered on the sewage farm was very different as compared to off the farm. Large populations of fecal associated bacteria occurred on the sewage farm and in the fenced plot (site 20) on the sewage farm. Populations of enterococci amounted to only 10 percent of the population of the fecal and total coliforms. The populations of bacteria present in soil collected from different sites on the sewage farm were not significantly different (5% level of significance). No coliforms or enterococci were isolated from the site off the sewage farm. Thus, irrigation of the sewage farm soils with sewage lagoon effluent contributed significantly to the numbers of fecal associated bacteria in the soils.

Core samples from two soil series on the sewage farm were examined to determine the distribution of total aerobic and total coliform bacteria in soil profiles. Off farm controls were also included. Differences between total aerobic bacteria on and off the farm were not significantly different (5% significance level) (Tables 7 and 8). However, there were substantial decreases in bacterial numbers with increasing soil depth. This probably was due to the nutrients available in the soil for the growth of the indigenous bacteria, and not due to leaching of these bacteria through the soil.

Coliform bacteria were substantially greater in numbers in the soil of the sewage farm than in the soil not treated with sewage (Tables 9 and 10). There were differences in numbers of bacteria between the two soil series obtained from the sewage farm. This difference may have been due to irrigation. The Rio Concho soil had been recently irrigated with sewage effluent; however, the San Antonio soil had not been irrigated for at least 3 weeks. The bacteria present in the San Angelo soil remained relatively constant for the first 30 to 40 cm of soil, but were not detected below this. Numbers of coliform bacteria in the Rio Concho soil decreased by a magnitude of 1.5 logs in the uppermost 10 cm of the soil. The population of coliform bacteria decreased slightly throughout the remainder of the soil cores.

Salmonella in Sewage, Seepage Creeks, Lagoons, Soil and the Concho River

A pathogenic bacterium commonly present in sewage and likely to be of public health significance is salmonella. Therefore, attempts were made to isolate salmonella from various locations on the sewage farm. *Salmonella* sp. were isolated from raw sewage, primary settling tank, all lagoons, in Seepage Creeks 1 and 2, and in the soil (Table 11). Best recovery of salmonella was accomplished by using tetrathionate broth without supplements using a 30 ml enrichment volume. Organisms were isolated from both the raw sewage and irrigation effluent. Organisms demonstrating the typical salmonella biochemical pattern were sent to the Houston City Health Laboratory for Somatic "O" and Flagellar "H" antigenic analysis. Serotype *Salmonella saint paul* 1, 4, 5, 12: e, h: 1, 2 was isolated from the raw sewage. Serotype *Salmonella panama* 1, 9, 12: 1, V: 1, 5 was isolated from the irrigation effluent. Biochemically presumptive *Salmonella* sp. were

TABLE 7. TOTAL AEROBIC BACTERIA PRESENT IN CORE SAMPLES OF THE SAN ANGELO SERIES SOIL ON THE SAN ANGELO SEWAGE FARM AND A FARM NOT RECEIVING SEWAGE

Sampling depth cm	sewage farm			farm		
	1	2	3	1	2	3
	1 x 10^4/g soil					
0 - 5	3,300	3,200	3,100	1,900	3,500	200
5 - 10	1,400	1,200	1,700	1,000	980	615
10 - 15	980	720	400	675	1,000	360
15 - 20	850	740	450	400	700	850
20 - 30	480	990	140	163	190	110
30 - 40	270	430	800	990	126	670
40 - 50	230	48	340	166	960	770
50 - 100	97	15	100	2,700	16	350
100 - 150	33	-	40	990	9	49

TABLE 8. TOTAL AEROBIC BACTERIA PRESENT IN CORE SAMPLES OF THE RIO CONCHO SERIES SOIL ON THE SAN ANGELO SEWAGE FARM AND A FARM NOT RECEIVING SEWAGE

cm	sewage farm			farm		
	1	2	3	1	2	3
	1 x 10^5/g soil					
0 - 5	3,800	280	>2,000	180	430	89
5 - 10	630	60	>2,000	280	150	59
10 - 15	400	40	2,000	94	65	31
15 - 20	330	13	150	99	48	74
20 - 30	9	8	7	280	35	32
30 - 40	10	11	3	66	15	11
40 - 50	6	7	9	20	8	8
50 - 100	3	10	2	23	2	6
100 - 150	11	2	2	14	35	6

TABLE 9. COLIFORM BACTERIA PRESENT IN CORE SAMPLES OF THE RIO CONCHO SERIES SOIL ON THE SAN ANGELO SEWAGE FARM AND A FARM NOT RECEIVING SEWAGE

Sampling depth cm	sewage farm			farm		
	1	2	3	1	2	3
	1 x 10^2/g soil					
0 - 5	5,000	1,500	2,700	<1	3	5
5 - 10	290	130	30	3	1	5
10 - 15	150	30	70	<1	<1	<1
15 - 20	90	20	20	<1	<1	<1
20 - 30	38	10	10	<1	<1	<1
30 - 40	33	25	15	<1	<1	<1
40 - 50	23	5	40	<1	<1	<1
50 - 100	20	10	10	<1	<1	<1
100 - 150	2	<1	<1	<1	<1	<1

Symbol < means less than

TABLE 10. COLIFORM BACTERIA PRESENT IN CORE SAMPLES OF THE SAN ANGELO SERIES SOIL ON THE SAN ANGELO SEWAGE FARM AND A FARM NOT RECEIVING SEWAGE

cm	sewage farm			farm		
	1	2	3	1	2	3
	1 x 10^2/g soil					
0 - 5	20	10	30	<1	14	2
5 - 10	20	95	16	1	1	1
10 - 15	20	60	15	1	<1	<1
15 - 20	<1	50	50	<1	<1	<1
20 - 30	<1	20	3	<1	<1	<1
30 - 40	<1	<1	1	<1	1	<1
40 - 50	<1	<1	<1	<1	<1	<1
50 - 100	<1	<1	<1	<1	<1	<1
100 - 150	<1	<1	<1	<1	<1	<1

Symbol < means less than

found in a 50 g soil sample from site 6 when enriched in TT broth. Although no salmonellae were isolated from the Concho River, their presence in the seepage creeks suggest that they were added to the river.

The isolation of Salmonella enteriditis serotype Saint Paul from both the raw sewage and lagoon effluents suggested that salmonella survived and passed through the system. Therefore, there was the potential for salmonella to be introduced into the Concho River. The load of fecal indicators in the seepage creeks and Concho River were essentially the same, and in spite of the small population of these indicators, salmonella was isolated. This suggests that in low numbers, fecal coliform-enterococci indicators are not reliable in determining the possibility of salmonella contamination.

Salmonella in Bovine Feces

Cattle are capable of being infected by Salmonella sp. and may shed the organism in their manure. The presence of salmonellae in sewage, water, and soil provides a source of the organisms for the cattle on the sewage farm to become infected. To determine if cattle, placed on the sewage farm, became infected with salmonella, the manure from 10 cattle was monitored from the day the cattle arrived on the farm. Fresh manure samples were taken at bi-weekly intervals for a total of 10 samplings. Salmonella was isolated from one animal before it was released on the farm.

Only one animal shed salmonella in its manure. It was not, however, the same animal that was infected before release on the farm. Manure from this animal only contained salmonella at one sampling. Therefore, the sewage farm did not increase shedding of Salmonella sp. in cattle feces.

TABLE 11. NUMBER OF SEWAGE, WATER AND SOIL SAMPLES THAT SALMONELLAE WAS ISOLATED FROM

Sample	No. Positive	Sample	No. Positive
Raw Sewage	1	Deep Well	0
Primary Settling Tank	5	Seepage Creek 1	1
Sewage Lagoon #1	5	Seepage Creek 2	1
Sewage Lagoon #2	5	Seepage Creek 3	1
Sewage Lagoon #3	5	Concho River Upstream	0
Sewage Lagoon #4	5	Concho River Downstream	0
Irrigation Effluent	1	Soil (Site 13)	1

Laboratory Studies with Bacteria

Introduction

The field investigations revealed that microorganisms from sewage effluent leached through soils of the sewage farm, but field data were not obtained to relate the leaching characteristics of a disease organism, salmonella, to that of common fecal bacteria. Therefore, laboratory experiments were initiated to measure the relative leachability of different bacteria added to soil. More than one soil was used to determine if there was an interaction between soils and bacteria on the leaching of bacteria.

Filter Selection

Before leaching experiments could be initiated, columns of soil had to be constructed in a manner that they could be leached without loss of soil. To accomplish this, filters had to be used on the bottom of the columns. Many filters were compared for their ability to pass bacteria, and to allow for sufficient suction on the soil for it to drain to field capacity. Three types of filters were tested: Nucleopore, teflon, and cellulose (Table 12). The teflon filters greatly impeded water movement. Because of this, the teflon filters were not suited for this experiment. The cellulose filters were fibrous filters like the teflon filters, but liquids could pass through them easily. The nucleopore filters consisted of a thin sheet of polycarbonate with the holes "punched" through the sheets. The 3 µm nucleopore filter was chosen for the soil column experiments because it allowed the greatest amount of liquid from the soil column to pass through when suction was applied. Also, only a small decrease in bacterial numbers occurred when the bacteria were passed through the 3 µm nucleopore filter (Table 13).

Adsorption

Bacteria are prevented from leaching through soils by the filtering action of the soil and also by adsorption of bacteria onto soil particles. Therefore, the adsorption of bacteria to soil particles greater than 1 µm in diameter was measured by adding bacteria to soil and separating the non-adsorbed bacteria by differential centrifugation. Adsorbed bacteria varied between 9 percent of the total applied bacteria for the Arenosa sand, to over 99.9 percent for the Beaumont clay (Table 14). There were differences in adsorption between bacterial species. The greatest differences occurred with the San Angelo soil; approximately 60 percent and 80 percent, respectively, of the *E. coli* and *S. typhimurium* were adsorbed. Increased adsorption for *S. typhimurium* also occurred for the Houston Black clay; approximately 90 percent and 98 percent, respectively, of the *E. coli* and *S. typhimurium* were adsorbed. Relatively small differences in bacterial adsorption occurred in the Arenosa sand and the Beaumont clay.

Bacterial cells normally range in size between 1.6 to 0.9 µm. Therefore, 1 µm soil particles were chosen to represent the minimum clay fraction that could easily be separated from the non-adsorbed bacteria. The suspension of soil and bacteria was centrifuged until all soil particles

TABLE 12. PERCENTAGE WATER REMAINING IN A SAN ANGELO SANDY CLAY LOAM COLUMN 1 cm IN DEPTH, AFTER DRAINAGE

Filter	Water content[a]
3 µm Nucleopore (polycarbonate)	26.8%
5 µm Nucleopore (polycarbonate)	32.6%
8 µm Selectron (cellulose)	29.5%
5 µm Millipore (teflon)	35.5%

[a]Percent water retained in soil against 1/3 atm. pressure was 27.4%

TABLE 13. DECREASES IN NUMBERS OF E. COLI WHEN AN INITIAL 10 ml ALIQUOT OF THE BACTERIA WERE PASSED THROUGH DIFFERENT TYPES OF FILTERS

Filter	Water content[a]
3 µm Nucleopore	1.4×10^6 (9.0×10^4)
5 µm Nucleopore	1.4×10^6 (2.9×10^5)
8 µm fibrous	5.5×10^5 (1.1×10^5)

[a]The initial number of bacteria was 1.5×10^6/ml (3.0×10^4) and the number in parenthesis is the standard deviation

TABLE 14. PERCENTAGE OF BACTERIA ADSORBED ONTO SOIL PARTICLES GREATER THAN 1 µm IN DIAMETER

Soil Type	E. coli	S. typhimurium
Arenosa	7 (7.5)[a]	11 (6.3)
San Angelo	63 (1.4)	82 (1.9)
Houston Black	90 (0.7)	98 (1.4)
Beaumont	99.9 (0.01)	99.9 (0.08)

[a]Standard deviations are in parenthesis

greater than 1 μm were pelleted. This procedure could be considered a differential centrifugation process, since the particle density for clay is approximately 2 g/cm^3, and the particle density for bacterial cells is approximately 1.08 g/cm^3.

The true adsorption values for these soils may have been somewhat lower than the measured values, especially the clay soils, due to possible adsorption of many of the finer clay particles to the bacteria. The bacteria may have become massive enough after adsorbing many small clay particles to centrifuge out with the bacteria adsorbed to the 1 μm particles. An increase in surface area and a larger net negative charge for the clay soils was probably responsible for the increased bacterial adsorption over that occurring for the sandy soils.

Evidence for finer clay materials adsorbing onto bacterial cells has been shown in transmission electron micrographs (Marshall 1971). These smaller clay fractions adsorbing onto the bacterial surfaces may simply move with the organism through the soil, not impeding the movement of the bacteria. This phenomenon might actually have promoted the movement of the bacteria, if a thin coat of the negatively charged particles prevented adsorption of the bacterium to the large particles by repulsive forces.

Bacterial adsorption increased with increasing clay content. This was not unexpected. In Marshall's review of sorptive interactions many investigators have reported increased bacterial adsorption with increasing clay content. The clay content appears to be much more important than the organic matter content, since the San Angelo and Houston Black soils contained the higher organic matter content, but did not adsorb the most bacteria.

A plausible explanation for the differences in bacterial adsorption in the same soil may be that the surface charges of the two bacterial species were different. Marshall (1971) has reported a distinct relationship between the amount of clay sorbed per unit area of cell surface and the nature of the surface ionogenic groups of the bacteria.

Bacterial Size

The size of bacteria probably has an effect on the movement of bacteria through a soil. Therefore, the size of the bacteria used in these investigations was measured with the light microscope and is illustrated in Figure 12. Escherichia coli was the largest bacterium, averaging 1.8 by 0.9 μm. Salmonella typhimurium and Salmonella enteriditis were similar in size, measuring 1.6 by 0.9 μm. Streptococcus fecalis was coccoidal in shape and measured 1 μm in diameter. A number of bacterial pairs were observed in the streptococcus suspension, but this may have been due to the procedure in preparing the organisms for observation.

Pore Size Distribution

The pore size distribution of soils would be expected to influence the quantity of bacteria that could leach through. The pore size distribution

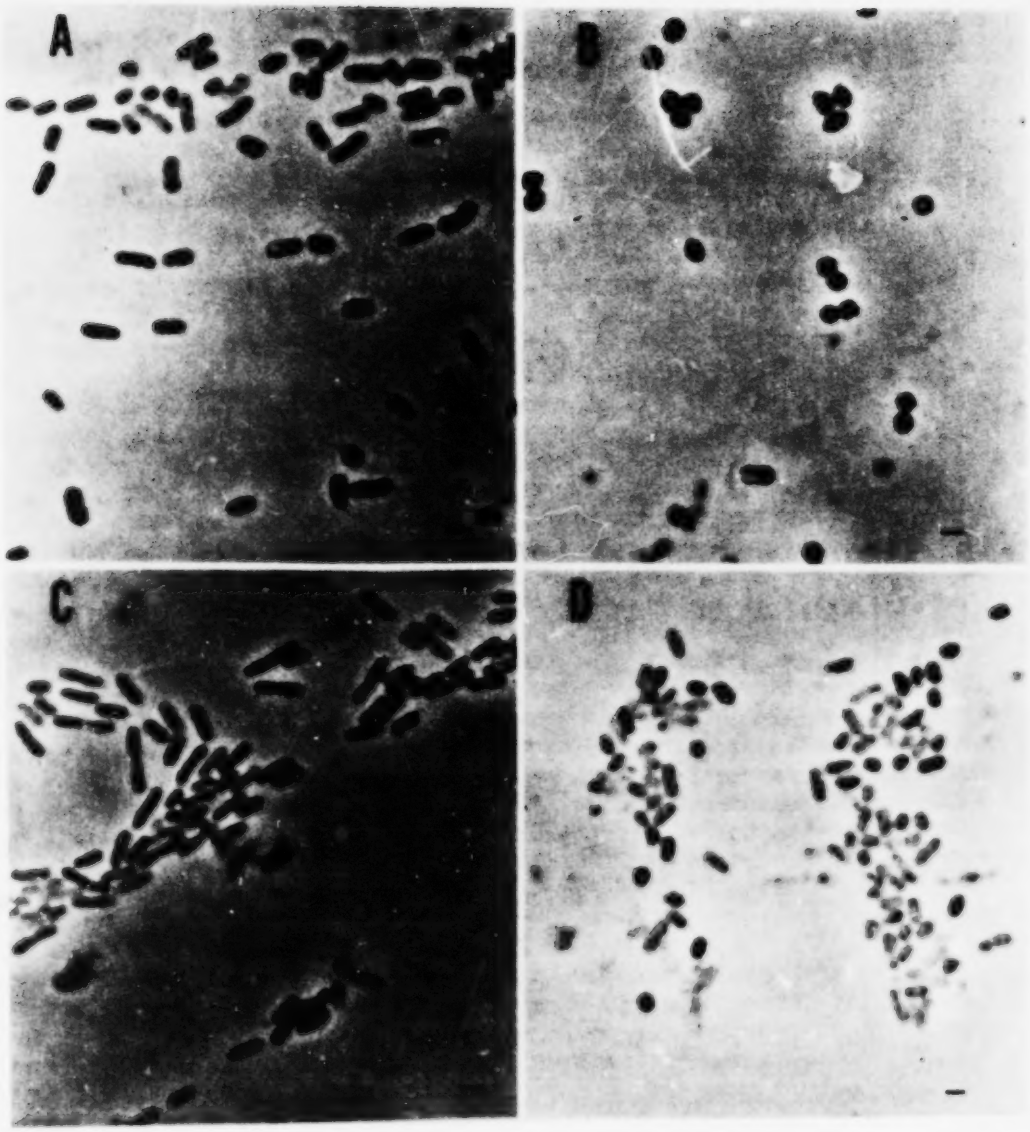

Figure 12. Light micrographs of the bacteria used for size determinations. A. Escherichia coli B. Streptococcus fecalis C. Salmonella enteriditis D. Salmonella typhimurium. Line represents 1 µm.

of the soils used in these investigations is shown in Table 3. The majority of the total pore space was comprised of pores greater than 12 µm in diameter. For most soils, less than 9 percent of the pore space was comprised of pores less than 3 µm. For example there were virtually no pore spaces less than 6 µm in diameter in the Arenosa sand. Therefore, there was little possibility of this soil filtering out single bacterial cells. Filtration of clusters of bacteria could have occurred if the clusters were relatively large. Krone (1958) has shown that filtration of bacterial clusters could be an important process in soils.

All of the soils contained a large fraction of pores having diameters of 50 to 12 µm that accounted for between 20 to 41 percent of the total pore volume of the soils. These larger pores would be the pores most conducive to leaching of bacteria. In all soils, except for the Arenosa sand, there was a fraction of the pore space, between 5 and 9 percent, that was comprised of pores smaller than the diameters of the bacteria. These smaller pores would impede the movement of even a single bacterium by filtration. This would represent case II filtration proposed by Krone. Therefore, the finer textured soils or the one with a greater percentage of pore space comprised of small pores would have the greatest potential to filter out bacteria.

Leaching Through Soils

Small differences were observed for the leaching characteristics of the four bacteria through the Arenosa loamy sand (Table 15 and Fig. 13). Approximately 5 percent of the bacteria passed through 5 cm of this soil. The numbers of bacteria, when leached through 15 cm columns of this soil, were reduced over 2 logarithmic units or 99.5 percent.

A two-phase curve could be seen in the plot of bacterial numbers in the leachate vs. soil depth (Figure 13). The first section of this curve was the relatively rapid decrease in bacterial numbers that occurred for each increment of soil in the first 5 cm. The second phase was a more gradual decrease in bacterial numbers per increment of soil. This phase began between 5 and 10 cm of soil.

Analysis between the proportions of bacteria that passed through this soil indicated that there were significant differences between the bacteria (Table 15). The leaching characteristics for E. coli and S. typhimurium were similar thoughout this soil. There were a few statistically significant differences between S. fecalis and the bacteria E. coli and S. typhimurium; however, at most soil depths there were no differences. More S. enteriditis than the other bacteria leached through the first 5 cm of this soil. Similar quantities of S. fecalis as well as S. enteriditis leached through 10 and 15 cm of soil.

For the 10 and 15 cm depths the leachability of S. fecalis and S. enteriditis were similar, and the leachability of S. typhimurium were similar. However, the effect per cm of soil height was the same between these two depths. This indicates that the differential rate of leaching for the organisms would have occurred before the 10 cm soil depth to allow

TABLE 15. THE PROPORTION OF ADDED BACTERIA THAT LEACHED THROUGH COLUMNS OF AN ARENOSA LOAMY SAND

Organism	Soil Depth						
	1 cm	2 cm	3 cm	4 cm	5 cm	10 cm	15 cm
E. coli[1]	.554 b	.329 b	.208 a	.084 a	.064 a	.004 a	.0013 a
S. fecalis	.906 c	.566 a,b	.216 a	.127 a	.060 a	.014 b	.0034 b
S. typhimurium	.249 a	.255 a	.123 a	.082 a	.013 b	.005 a	.0006 a
S. enteriditis	.987 c	.698 b	.653 b	.313 b	.164 b	.007 a	.0038 b
	(.347)[2]	(.209)	(.273)	(.111)	(.060)	(.005)	(.0016)

[1]Refer to table 3 for the quantity of bacteria added to each column. Values, in columns, having a letter in common were not significantly different at the 5% level

[2]The standard deviation is in parenthesis

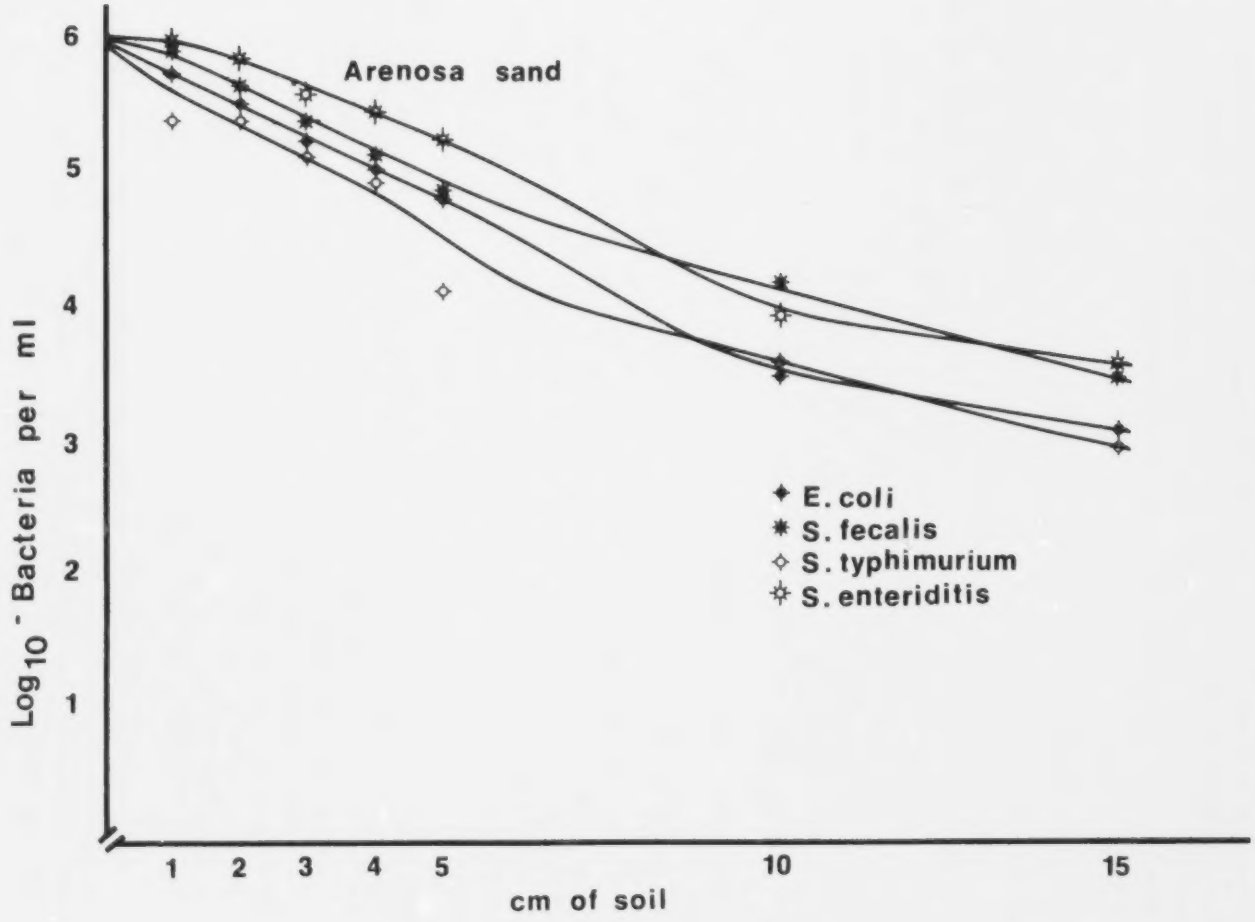

Figure 13. The effect of soil column height on the number of bacteria per ml of leachate.

for the separation at the 10 cm depth. A trend for this to happen occurred in the first 5 cm of soil.

There were greater differences in the leaching characteristics of the bacteria through the San Angelo sandy clay loam than occurred with the Arenosa sand (Table 16 and Figure 14). Only 0.8 percent of the bacteria passed through 5 cm columns of this soil. The bacterial numbers were reduced over 5.5 logarithmic units, or 99.999 percent, by passage through 15 cm columns.

A three-phase curve for E. coli, S. enteriditis, and S. fecalis could be seen in the plot of bacterial numbers in the leachate vs. soil depth (Figure 14). The first phase could be considered the relatively large reduction in numbers from that initially applied to that present in the leachate from the 1 cm column depths. This reduction was most apparent in the curve for E. coli. The second phase was considered the relatively slower linear decrease in bacterial numbers for the 1 and 5 cm depths. In comparison of this soil to the previous one, a more rapid decrease in numbers of bacteria was observed for the first 5 cm of the San Angelo soil. Bacterial numbers decreased at about the same rate between the third phase of the San Angelo soil and the first phase of the Arenosa sand.

The leaching characteristics for S. typhimurium, S. enteriditis, and S. fecalis were all similar for most of the soil depths. The decrease in numbers of E. coli for the first 5 cm of soil was substantially greater than the other bacteria. The reason for this phenomenon was not determined; however, it was not due to the size of the bacteria since all the bacteria were about the same size. At the 15 cm depth, the numbers of S. enteriditis and E. coli present in the leachate were not statistically different. However, the numbers of S. typhimurium and S. fecalis in the leachate from 15 cm columns were significantly greatly than the other two bacteria.

Almost all of the bacteria were retained in the first few cm of the Houston Black clay. The rate of decrease of bacteria in the leachate was very rapid for all of the bacteria (Table 17 and Figure 15). Escherichia coli, S. fecalis, and S. typhimurium were not detected in the leachate from columns longer than 2 cm. However, S. enteriditis was detected in the leachate from all column lengths with three organisms per 10 ml of leachate present from 15 cm columns. The population of S. fecalis and S. enteriditis were also similar for the first 2 cm of soil. However, S. fecalis was not detected beyond this point.

A two-phase curve could only be seen in the plot of the numbers of S. enteriditis in the leachate vs. soil depth (Figure 15). All the other bacteria were eliminated so quickly from the soil that a straight line was observed for the bacterial decreases.

Beaumont clay is a dense, poorly structured soil with very low permeability. Because of the tortuous path taken by the liquid, the large fraction of small pores, and the high adsorption rate, no bacteria passed through even 1 cm of this soil.

TABLE 16. THE PROPORTION OF ADDED BACTERIA THAT LEACHED THROUGH COLUMNS OF A SAN ANGELO SANDY CLAY LOAM

Organism	Soil Depth						
	1 cm	2 cm	3 cm	4 cm	5 cm	10 cm	15 cm
E. coli	.0141a	.0017a	.0007a	.0002a	.00001a	.000001a	3.7×10^{-6}a
S. fecalis	.2237b	.0603c	.0303c	.0038b	.0032c	.00004a	7.1×10^{-5}c
S. typhimurium	.4226c	.0576c	.0162b	.0066c	.0009b	.00009a	1.0×10^{-5}b
S. enteriditis	.1229b	.0223b	.0109b	.0032b	.0008b	.00003a	3.1×10^{-6}a
	(.1711)[2]	(.0286)	(.0107)	(.0026)	(.0012)	(.00004)	(4.4×10^{-6})

[1]Refer to table 3 for the quantity of bacteria added to each column. Values, in columns, having a letter in common were not significantly different at the 5% level

[2]The standard deviation is in parenthesis

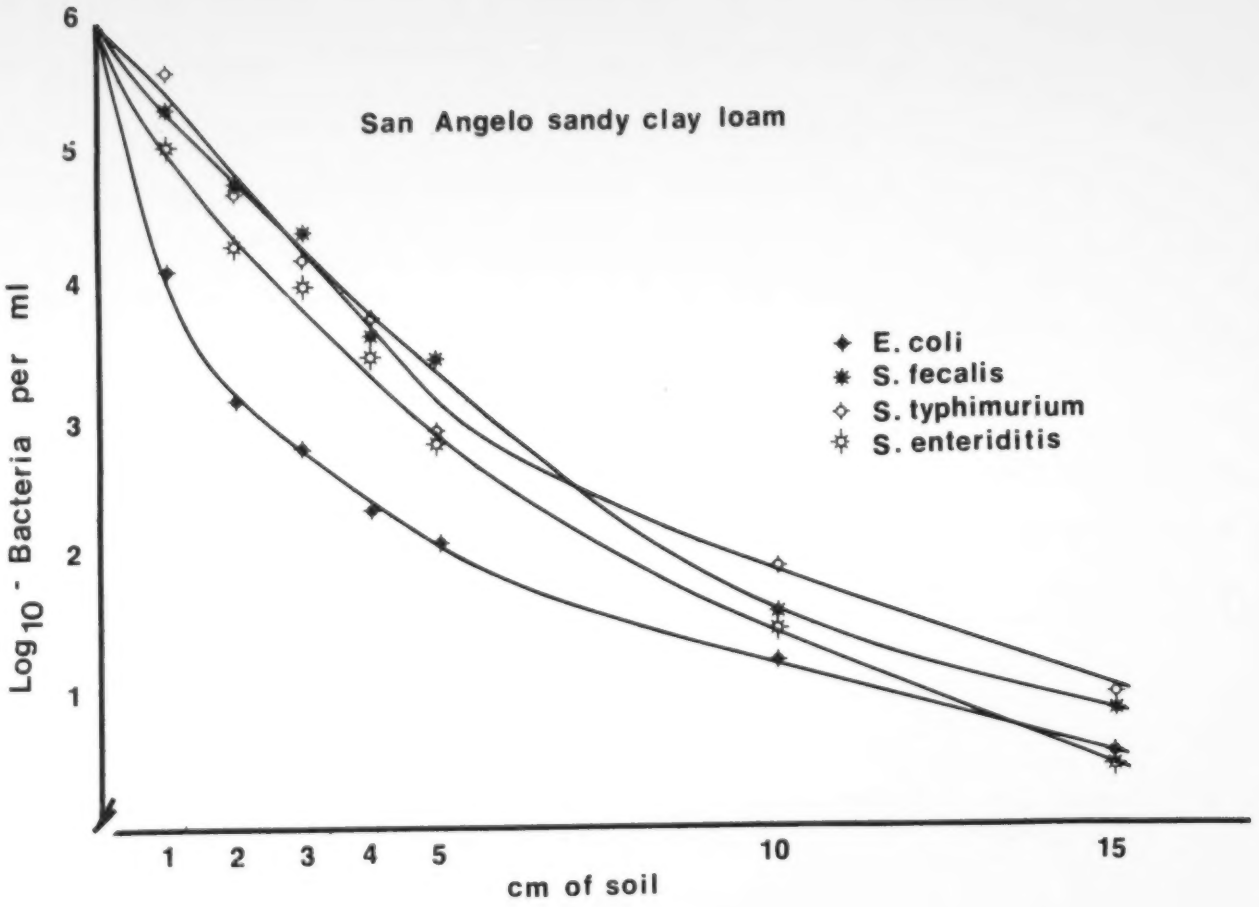

Figure 14. The effect of soil column height on the number of bacteria per ml of leachate.

TABLE 17. THE PROPORTION OF ADDED BACTERIA THAT LEACHED THROUGH COLUMNS OF A HOUSTON BLACK CLAY

Organism	Soil Depth						
	1 cm	2 cm	3 cm	4 cm	5 cm	10 cm	15 cm
E. coli[1]	.0051 a	7.5×10^{-6} a					
S. fecalis	.0655 b	.0053 b					
S. typhimurium	.0033 a	---[2] d					
S. enteriditis	.1038 c	.0966 c	.0027	.0006	.00003	1.0×10^{-6}	1.4×10^{-7}
	(.0442)[3]	(.0472)	(.0003)	(.0003)	(.00002)	(3.8×10^{-7})	(7.0×10^{-8})

[1]Refer to table 3 for the quantity of bacteria added to each column. Values in columns having a letter in common were not significantly different at the 5% level.

[2]No bacteria were detected in the leachate

[3]Standard deviation is in parenthesis

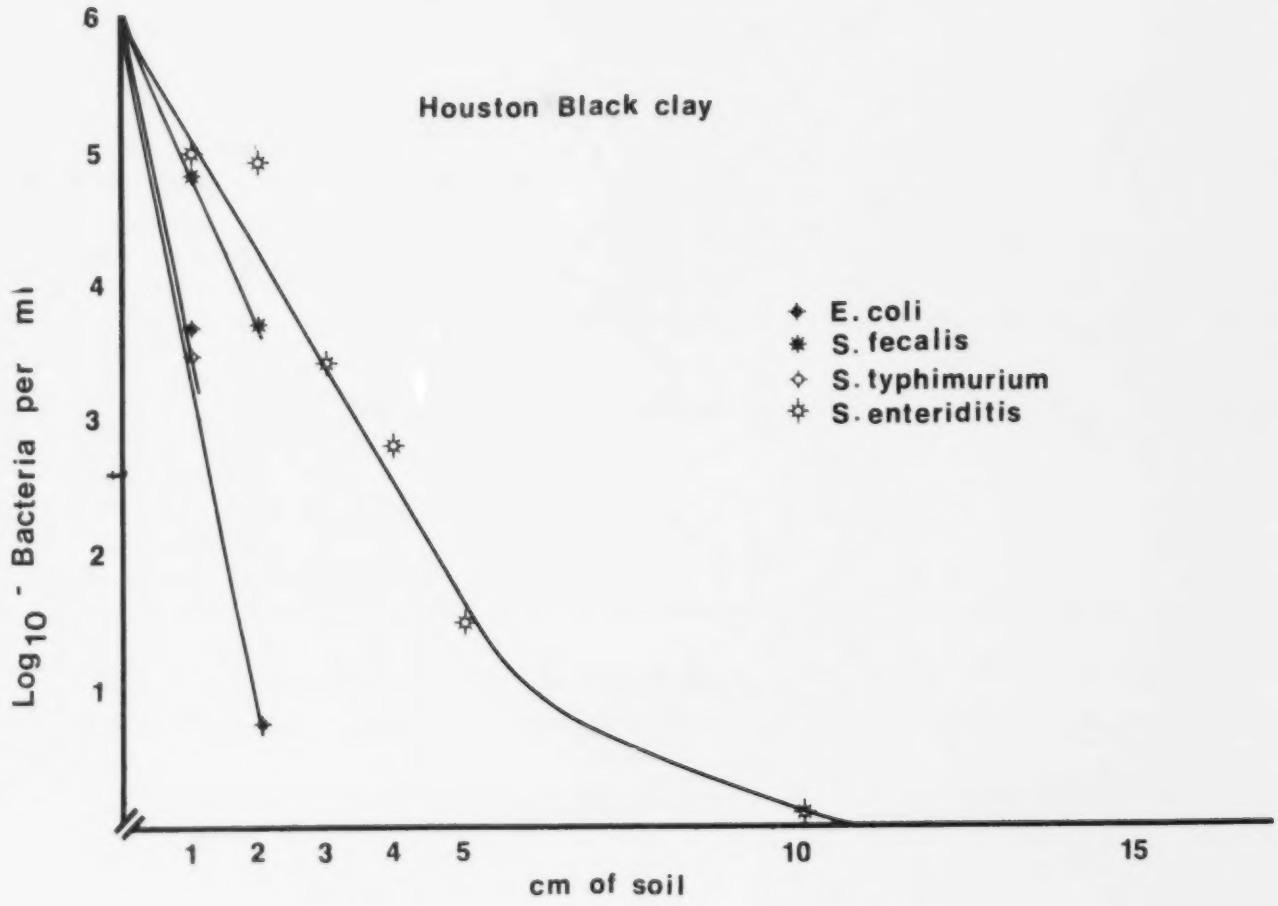

Figure 15. The effect of soil column height on the number of bacteria per ml of leachate.

There were significant differences in bacterial movement through the four soils used in this investigation. The sandy soil retained the fewest bacteria; whereas, the Beaumont clay retained all of the bacteria. The retention capacity of the soils increased with increasing clay content. The order of increasing retention was: Arenosa loamy sand, San Angelo sandy clay loam, Houston Black clay, and Beaumont clay.

Adsorption was probably an important factor in bacterial retention in the clay soils. The hydraulic conductivity in these soils was relatively low. Therefore, the bacteria moving through such soils would have a greater opportunity to interact with the soil particles. As the adsorption results indicated (Table 14), under conditions where the bacteria come in contact with many soil particles, a very large percentage of the bacteria could be adsorbed.

The removal mechanisms for bacteria passing through soils may selectively remove a portion of the bacterial population. This was indicated by the nonlinear plots of column height and population of bacteria in the leachates (Figures 13, 14, and 15). A theory proposed by Gerba and Lance (1976), for viruses, was that the surface soil layers remove the majority of the highly charged viral particles and the remaining more neutrally charged viruses, could pass further into the soil. This theory may also apply to the adsorption of bacteria, since bacterial cells have charged surfaces (Hattori and Hattori 1976, Hattori 1973, and Marshall 1971).

Soil structure has a large influence on the leaching of bacteria. The Houston Black clay is a Vertisol, and, in field condition, large cracks can occur during periods of dryness. The columns from these experiments were packed with dry soil and did not contain large cracks. Therefore, the results reported here have demonstrated the maximum retention of the bacteria, because the bacteria had to travel through the soil pores rather than escape through large cracks or cleavage plains.

The overall results indicate that the bacteria basically behaved similarly in their leaching characteristics through soils. However, slight differences in leaching characteristics did occur for the different bacteria and were not related to the size of the bacteria. For any soil type, a particular organism could not be predicted to leach through the soil more readily than another. In these experiments S. fecalis rather than E. coli behaved most similarly to salmonella and may be the better indicator for the leaching rate of salmonella through soils.

Distribution of Bacteria in Columns

Because salmonella is of particular public health significance, it was used as the test organism to determine the distribution of bacteria within leached soil columns, to determine the effect of salts in the leaching solution on the movement of bacteria through soil columns, to determine the rate at which bacteria move through soil columns relative to the movement of the leaching solution, and to determine the effect of leaching a soil more than once with a bacterial suspension.

To determine the distribution of viable salmonella in soil columns after an inoculum was passed through, columns were sectioned and the bacteria in each section were enumerated. To accomplish this, 15 cm columns were constructed as in the leaching experiments, and 75 ml of a salmonella suspension was leached through the soils. The bacteria in the 0-3, 3-5, 5-10 and 10-15 cm sections were enumerated.

The distribution of bacteria in sections of Arenosa sand ranged from 2.6×10^5 to 8.2×10^3 bacteria per g of soil (Table 18). The percentage of the applied bacteria in each section was 26, 6.5, 1.9, and 0.8 percent, respectively for the 0-3, 3-5, 5-10 and 10-15 cm sections. The numbers of bacteria in the San Angelo soil ranged from 1.1×10^5 bacteria per g of soil in the 0-3 cm section, to 3.0×10^2 bacteria per g of soil in the 10-15 cm section. Percentages of applied bacteria retained in the 0-3, 3-5, 5-10, and 10-15 cm sections of the soil, were, respectively, 11, 0.12, 0.02 and 0.03. Bacteria were only present in the uppermost section of the Houston Black clay. Eleven percent of the total applied bacteria were recovered in this segment.

The results from enumerating the bacteria present in the soil sections indicated similar trends to the results obtained by determining the total numbers of bacteria present in the leachate passed through soil columns of various heights. That is, the first increments of soil removed proportionately more bacteria than soil located further down the columns.

The detectable salmonella present in the columns were considerably less than expected, since the recovery of salmonella from columns varied between 26 to 11 percent of the cells added. This phenomenon is probably best explained by the die-off of the bacteria, since the bacteria were usually in contact with the soil for a period of at least 1 hour.

Effect of Salts on Leaching

Variations in the retention and the leachability of bacteria may be affected by the ionic strength and the nature of the ions in the transport liquid (Cooper et al. 1975). Therefore, fluctuations in the leachability of salmonellae using different salts as the transport medium were compared to saline solution. Zohar et al. (1971) also reported that differences in the salt concentrations of the transport medium affected the leachability of bacteria.

There were no statistically significant differences (0.05 level) between the salts on leachability of salmonella through the two soils (Table 19). Our results indicate that the ionic strength of the transport liquid does not greatly affect bacterial numbers leached through the Arenosa loamy sand or the San Angelo clay loam.

TABLE 18. TOTAL NUMBERS OF SALMONELLA TYPHIMURIUM IN SECTIONS OF 15 cm SOIL COLUMNS AFTER LEACHING[a]

Soil depth	Soils		
	Arenosa loamy sand	San Angelo sandy clay loam	Houston Black clay
0 - 3 cm	2.6×10^5 (1.5×10^4)	1.1×10^5 (3.0×10^3)	1.1×10^5 (1.6×10^4)
3 - 5 cm	6.2×10^4 (1.7×10^4)	1.2×10^3 (2.4×10^2)	N.D.[b]
5 -10 cm	1.9×10^4 (7.0×10^2)	2.3×10^2 (1.0×10^2)	N.D.
10 -15 cm	8.2×10^3 (1.1×10^3)	3.0×10^2 (1.0×10^2)	N.D.

[a]Standard deviations are present in parenthesis

[b]N.D. -- not detected

Rate of Appearance in Leachate

Bacteria present in each 2 ml increment of leachate passed through 5 cm soil columns were enumerated to determine the rate bacteria pass through a soil relative to the rate of the leaching solution. The initial 2 ml increment from the Arenosa sand contained approximately 200 bacteria per ml, but the third increment contained 2350 bacteria per ml (Figure 16). As the soil dried, bacterial numbers decreased very rapidly. Of the initial 25 ml of liquid applied to the soil, about 21 ml leached through the soil. These results provide evidence that the soil solution passes through many pores that the bacteria are restricted from passing through.

Bacterial leaching through the San Angelo soil behaved similarly. The first increment of leachate contained about 73 bacteria per ml, and the third increment contained 166 bacteria per ml (Figure 16).

A cumulative curve of the numbers of bacteria in the leachate demonstrates that after the third increment for the Arenosa sand, and after the fourth increment for the San Angelo soil, the leaching rates for the bacteria were relatively constant (Figure 17). The slopes of the log of bacteria vs soil depth show that more bacteria passed through the Arenosa sand per increment of leachate than through the San Angelo clay loam.

TABLE 19. EFFECTS OF DIFFERENT SALT SOLUTIONS ON THE LEACHING OF *SALMONELLA TYPHIMURIUM* THROUGH 15 cm COLUMNS OF SOILS[a]

Salt solutions	Bacteria per ml[b]	
	Arenosa sand	San Angelo clay loam
Saline (0.31 N NaCl)	1.3×10^4	9.3×10^2
Salt solution (0.31 N $CaCl_2$ and NaCl - equimolar)	2.4×10^4	2.6×10^3
Salt solution (0.15 N $CaCl_2$ and NaCl - equimolar)	1.7×10^4	7.1×10^2
Salt solution (0.07 N $CaCl_2$ and NaCl - equimolar)	1.3×10^4	2.3×10^3
Phosphate buffer (0.01 M K_2HPO_4)	1.3×10^4	8.2×10^2

[a]The F test (0.05 level) indicated there were no statistically significant differences, between leaching solutions, on the leaching of salmonella

[b]Initial bacterial concentration of 10^6 organisms per ml was used

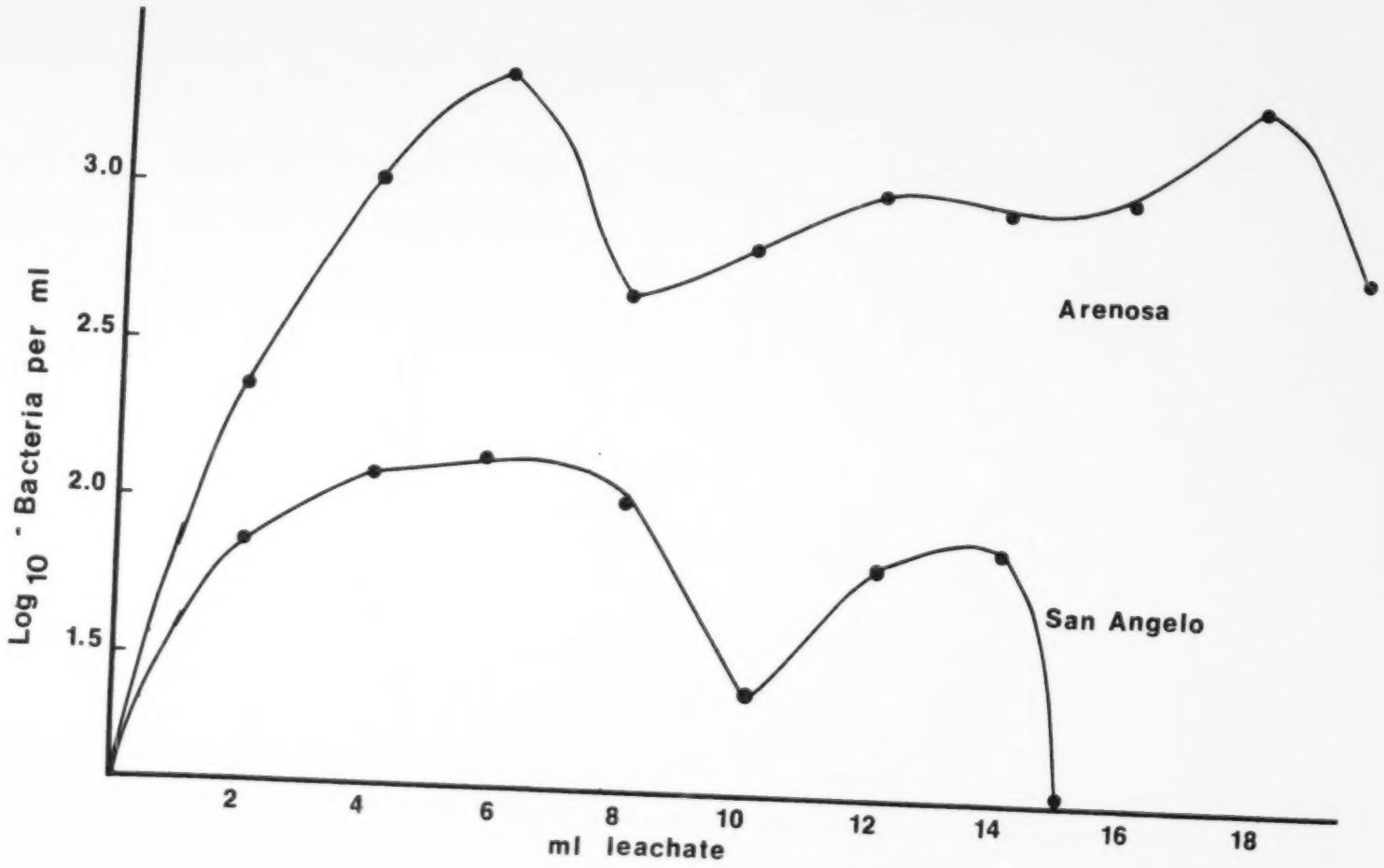

Figure 16. Number of bacteria present in consecutive 2 ml increments of leachate.

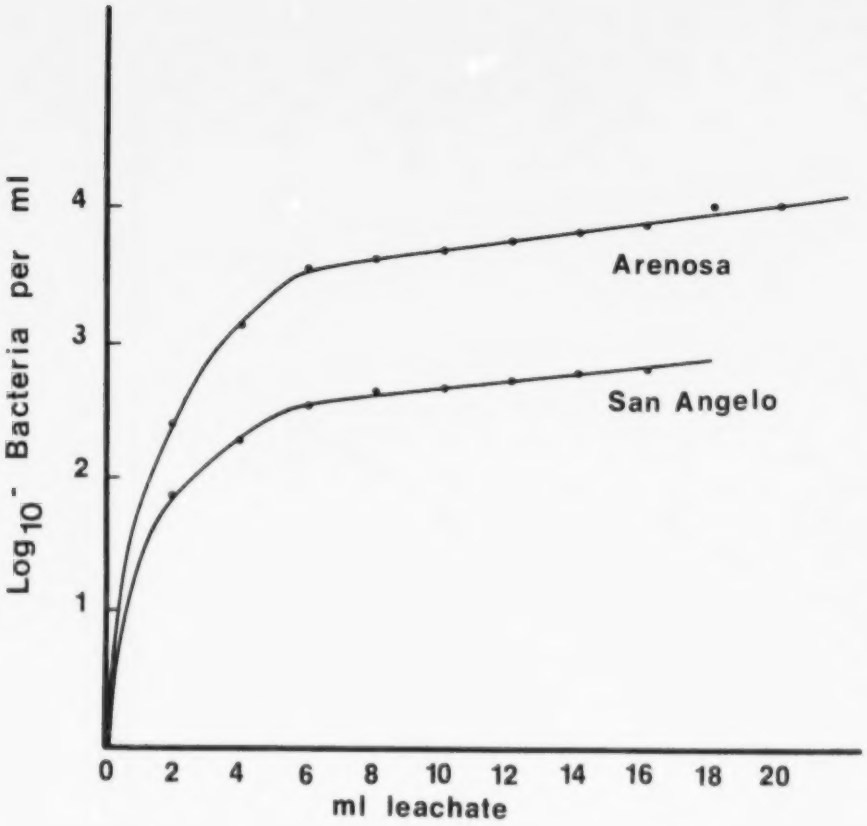

Figure 17. Cumulative numbers of bacteria present in consecutive 2 ml increments of leachate.

Saturation of Soil with Bacteria

Four increments of bacteria were passed through columns of three soils to determine the effects of consecutive additions of bacteria on the retention of the bacteria. The quantity of salmonella passing through Arenosa or San Angelo soils with each new inoculation remained relatively constant (Figure 18). A decrease in numbers of bacteria from 1×10^5 to 5×10^4 per ml of leachate was observed from the third to the fourth inoculation of the Arenosa soil. The numbers of bacteria that passed through 5 cm, San Angelo columns increased steadily for all four increments, from 7.5×10^2 bacteria per ml for the first increment, to 2.4×10^3 bacteria per ml in the fourth increment. Numbers of bacteria that passed through 1 cm of Houston Black clay increased on addition of the first three increments of bacteria, from 6.1×10^4 bacteria per ml, to 1.5×10^5 bacteria per ml. The fourth increment, probably due to clogging of pores by previously added bacteria, resulted in a rapid decrease in numbers of bacteria to 7.9×10^2 bacteria per ml.

These three soils reacted differently to repeated additions of bacteria. A possible explanation for the differences between soils is that the pores in the Arenosa sand were large enough to allow continued bacterial leaching without retaining most of the bacteria. In the fourth increment, a noticeable decrease of bacteria occurred, probably due to the bacteria clogging some of the smaller pores. The increases observed in the San Angelo soil were probably due to much of the water passing through pores that were too small for the bacteria. Repeated inoculations provided more water to complete leaching of nonadsorbed bacteria. The reason for the increase of bacteria passing through the Houston Black clay with addition of the second and third increments of bacteria, was probably the same as for the San Angelo soil. The low numbers of bacteria leaching through the soil in the fourth increment was probably due to clogging of soil pores by bacteria or to the dispersion of aggregates by the sodium present in the transport leaching solution.

Field Study on Viruses

Viruses were not detected in water or soil samples collected at various locations on the sewage farm. However, this does not mean that viruses were not present, only that the technique used on the water samples for these investigations was not sensitive enough to detect fewer than 10 viral particles per ml. Passage of the viruses through the millipore filters to remove bacteria probably further reduced the likelihood of detecting viruses. For example, we found that passage of Reovirus through a .45μ millipore filter reduced the population from 1×10^5 to 1×10^3 per ml. To quantitate the population of virus in the sewage waters and soils would have required concentration methods not available in our laboratory. Therefore, we directed our efforts to determining the leachability of viruses through columns of soil in the laboratory.

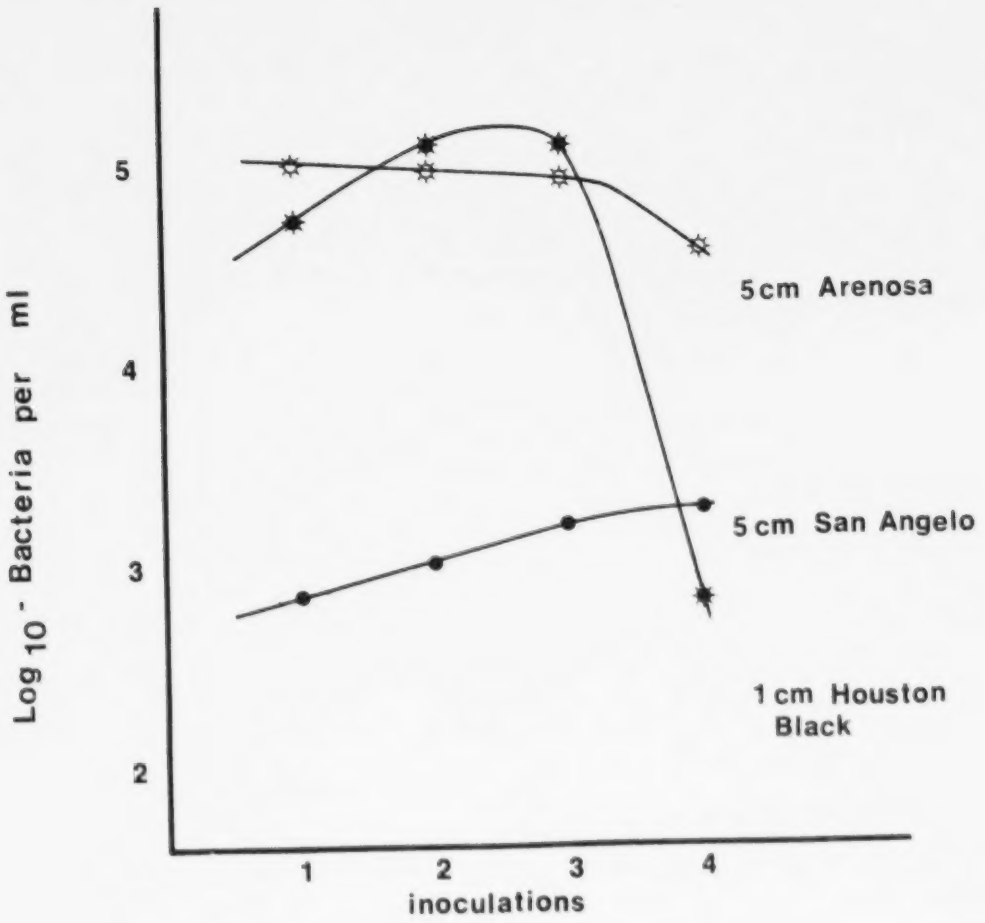

Figure 18. Bacteria present in the leachate after four consecutive inoculations of Salmonella typhimurium.

Laboratory Studies with Viruses

Analyses on the leachates collected from columns of a San Angelo sandy clay loam, ranging between 1 cm and 15 cm in length, revealed that Reovirus 3 was leached through some of the columns (Tables 20 and 21). There does not appear to be any difference in leaching of the virus whether saline or water was used as the eluate. However, there was a large amount of variability between columns as to the presence or absence of the virus in the leachate. Oftentimes one replication contained viral particles in the leachate and the other replication did not. Even some fractions of leachate from a column contained the virus but other fractions did not.

Leaching of Reovirus 3 through columns of a Houston Black clay was similar to its leaching through the San Angelo soil (Tables 22 and 23). These column studies prove that viruses can be leached through at least 15 cm of these soils when the soils have been prepared in a manner that would minimize leaching; the columns were filled with screened soil and were uniformly packed. This eliminated large pores and cracks normally present in soil in the field. The San Angelo soil is one of the main soil series on the San Angelo sewage farm.

The results of the assays used to determine if viruses were retained in the soil columns after passage of the eluate were inconsistent (Tables 24 and 25). In only a single instance was virus detected in the San Angelo soil. However, the frequency of detecting virus in the Houston Black soil taken from the leached columns was much greater than for the San Angelo soil. Viruses were not detected in the Houston Black soil taken from the 3 and 4 cm columns.

It seems reasonable that if the viral particles were not present in the leachate they should have been isolated from the soil. Yet in most instances this did not occur for the San Angelo soil (Tables 20, 21 and 24). Therefore, in some way the San Angelo soil must have inactivated or adsorbed many of the virus particles. This phenomenon only occurred for the 2 cm column of Houston Black soil that was leached with water. Apparently the San Angelo soil was much more effective in adsorbing or inactivating the virus. Both soils were calcareous, but the Houston Black soil contained substantially more clay and organic matter than the San Angelo soil. The time required for the eluate to leach through the San Angelo soil was less than for the Houston Black soil. The reason for the inactivation of virus in the San Angelo soil needs to be determined using quantitative methods.

PARASITOLOGICAL STUDIES

Detection of Possible Human Parasites in Sewage

The number of possible human parasites detected in raw sewage was unusually high (Table 26). The numbers of Entamoeba histolytica, E. coli and G. lamblia cysts were extremely variable from one month sample to the next, but E. histolytica was detected in almost every sample. Giardia

TABLE 20. PRESENCE OF VIRUS IN COLLECTIONS OF LEACHATE FROM GLASS COLUMNS FILLED WITH A SAN ANGELO SANDY CLAY LOAM AND REPETITIVELY LEACHED WITH PHYSIOLOGICAL SALINE

Column height cm	Number of collection	Quantity of saline added[a] ml	Presence of virus Replicate 1	2
1	1	5	-	+
	2	5	-	-
2	1	5	-	+
	2	5	+	+
3	1	5	-	-
	2	5	+	-
4	1	5	-	-
	2	5	-	-
	3	5	-	-
6	1	10	-	-
	2	5	-	-
	3	5	-	-
10	1	15	+	+
	2	5	-	+
	3	5	+	-
15	1	20	-	+
	2	5	-	-
	3	5	-	+

[a]First increment of liquid added was the quantity required to obtain a leachate. An additional 5 ml of liquid was added to the column for each additional collection

TABLE 21. PRESENCE OF VIRUS IN COLLECTIONS OF LEACHATE FROM GLASS COLUMNS FILLED WITH A SAN ANGELO SANDY CLAY LOAM AND REPETITIVELY LEACHED WITH WATER

Column height cm	Number of collection	Quantity of water added[a]	Presence of virus Replicate 1	Replicate 2
1	1	5	+	-
	2	5	+	-
2	1	5	-	+
	2	5	+	+
3	1	5	-	+
	2	5	-	+
4	1	5	-	-
	2	5	-	-
	3	5	+	+
6	1	10	-	-
	2	5	+	-
	3	5	-	+
10	1	15	+	-
	2	5	b	+
	3	5	+	+
15	1	20	-	-
	2	5	-	+
	3	5	+	+

[a]First increment of liquid added was the quantity required to obtain a leachate. An additional 5 ml of liquid was added to the column for each additional collection

[b]Sample not collected

TABLE 22. PRESENCE OF VIRUS IN COLLECTIONS OF LEACHATE FROM GLASS COLUMNS FILLED WITH A HOUSTON BLACK CLAY AND REPETITIVELY LEACHED WITH PHYSIOLOGICAL SALINE

Column height cm	Number of collection leaching	Quantity of saline added[a] ml	Presence of virus Replicate 1	2
1	1	5	-	+
	2	5	-	-
2	1	5	+	+
	2	5	+	-
3	1	5	[b]	-
	2	5	+	-
4	1	5	-	-
	2	5	+	[b]
	3	5	+	+
6	1	5	+	+
	2	5	+	+
	3	5	-	-
10	1	15	-	-
	2	5	-	-
	3	5	-	+

[a] First increment of liquid added was the quantity required to obtain a leachate. An additional 5 ml of liquid was added to the column for each additional collection

[b] Sample not collected

TABLE 23. PRESENCE OF VIRUS IN COLLECTIONS FROM GLASS COLUMNS FILLED WITH A HOUSTON BLACK CLAY AND REPETITIVELY LEACHED WITH WATER

Column height cm	Number of leaching	Quantity of water added[a]	Presence of virus Replicate	
			1	2
1	1	5	-	-
	2	5	-	-
2	1	5	-	-
	2	5	-	-
3	1	5	+	+
	2	5	+	-
4	1	5	+	+
	2	5	b	b
	3	5	+	+
6	1	10	b	-
	2	5	+	-
	3	5	-	b
10	1	15	-	+
	2	5	+	-
	3	5	+	+

[a]First increment of liquid added was the quantity required to obtain a leachate. An additional 5 ml of liquid was added to the column for each additional collection

[b]Sample not collected

TABLE 24. PRESENCE OF VIRUS IN SOIL COLLECTED FROM COLUMNS OF A SAN ANGELO SANDY CLAY LOAM THAT WERE INOCULATED WITH VIRUS AND LEACHED WITH PHYSIOLOGICAL SALINE OR WATER

Column height cm	Position in column	Saline Replicate 1	Saline Replicate 2	Water Replicate 1	Water Replicate 2
1	entire	a	a	a	a
2	entire	-	-	-	-
3	entire	-	-	-	-
4	entire	-	-	-	-
6	entire	a	a	a	-
10	top	a	a	a	-
	middle	a	a	a	-
	bottom	a	a	a	-

[a]Sample not analyzed or data missing

TABLE 25. PRESENCE OF VIRUS IN SOIL COLLECTED FROM COLUMNS OF A HOUSTON BLACK CLAY THAT WERE INOCULATED WITH VIRUS AND LEACHED WITH PHYSIOLOGICAL SALINE OR WATER

Column height cm	Position in column	Saline Replicate 1	Saline Replicate 2	Water Replicate 1	Water Replicate 2
1	entire	+	+	+	+
2	entire	+	-	-	-
3	entire	-	-	-	-
4	entire	-	-	-	-
6	top	-	-	+	+
	middle	+	-	+	+
	bottom	-	-	+	+
10	top	+	+	-	+
	middle	+	+	+	+
	bottom	+	+	+	+

lamblia and E. coli were detected in over half of the samples. Generally, E. histolytica and G. lamblia were the most prevalent parasites detected. Fewer parasites were detected during June and September, but this may have been due to chance because of the small samples collected, rather than a seasonal trend.

The population estimates given in Table 26 are above what would be normally expected in sewage and are partially due to the sampling method. The sensitivity of the hemocytometer method has definite limitations as a quantitative tool. Using a 25 to 1 concentration, the detection of only one organism indicated a density of 50 parasites per ml of sample. The concentration of larger volumes of sewage would have provided a more representative sample but other methods of filtering and concentration would have to be used.

Although materials identified as possible human parasites fitted existing descriptions, the nuclear elements of many protozoan cysts could not be clearly observed. Also, suspected protozoan cysts (eg. E. histolytica) did not always appear exactly as those seen when examining fresh feces from infected humans. It is possible that at least some of the materials identified as protozoan cysts could have been spores or other structures which resembled cysts.

Table 26 includes only those parasites which were consistently observed in raw sewage. Strongyloides sp. larvae, unidentified nematode eggs, Taenia sp. eggs, and unidentified trematode eggs were also detected by direct examination of concentrated raw sewage, but these parasites did not consistently show up when using hemocytometer counting grids. The eggs of other relatively common helminths, such as pinworm and hookworm, were not detected. Perhaps the membranes enclosing the eggs of some helminths, like hookworms and pinworms, were more delicate than those of the helminths detected in this study and may have ruptured under osmotic stress when placed in sewage wastewater. It is likely that pinworm infections were present in the local population at San Angelo.

Possible human parasites in the primary settling tank were also detected. Entamoeba histolytica, E. coli, and Giardia sp. were detected in wet mounts from samples taken any month from August of 1975 to January of 1976 except for September and November of 1975 and June of 1976. No samples were taken for February, March and May of 1976 because of shutdown of the primary settling tank to accommodate ongoing new construction at the sewage farm. Attempts to use the hemocytometer method on samples was generally unsatisfactory because of the amount of debris present. Therefore, only a qualitative examination was possible. Entamoeba histolytica and Giardia sp. were observed in about 1 out of 40 wet mounts from these samples and E. coli in approximately 1 out of 30 wet mounts. Ascaris sp. and Strongyloides sp. were not detected in these samples, but a Taenia sp. egg was found in the October 1975 sample.

No possible human parasites were detected in fluid samples from storage lagoons or in irrigation water from storage lagoons. It is possible that because of their large size, many of the human parasites settled out in the

TABLE 26. NUMBER OF FOUR POSSIBLE HUMAN PARASITES IN RAW SEWAGE ENTERING THE SEWAGE TREATMENT PLANT DURING 1975 AND 1976

Sampling Time	Entamoeba histolytica	Entamoeba coli	Ascaris sp.	Giardia sp.
	No. per ml			
June '75	60	30	0	0
July '75	13	20	80	80
Aug. '75	30	0	0	80
Sept. '75	20	0	0	80
Oct. '75	25	0	25	0
Nov. '75	50	50	0	25
Dec. '75	15	25	0	50
Jan. '76	30	30	0	25
Feb. '76	18	20	0	40
Mar. '76	30	20	0	40
Apr. '76	50	20	0	30
May '76	0	0	0	0
June '76	0	0	0	30

TABLE 27. RELATIVE ESTIMATES OF FOUR POSSIBLE HUMAN PARASITES IN SLUDGE

Sampling Time	Entamoeba histolytica		Entamoeba coli		Ascaris sp.		Giardia sp.	
	Surface	15 cm	Surface	15 cm	Surface	15 cm	Surface	15 cm
Jan. '76	1/40[a]	1/10	1/20	1/20	0	1/80	1/27	1/10
Feb. '76	1/40	1/20	0	0	0	0	0	1/40
Mar. '76	-	-	-	-	-	-	-	-
Apr. '76	0	1/40	1/20	1/20	0	1/36	1/40	1/18
May '76	-	-	-	-	-	-	-	-
June '76	0	1/27	0	0	0	0	0	0

[a]The number of positive wet mounts per number of wet mounts examined

primary settling tank with the sludge or sediments in the storage lagoons. If substantiated, this finding could be important in developing methods for the treatment of human sewage to remove human parasites.

Detection of Possible Human Parasites in Sludge

Although we were not able to quantify the number of possible human parasites in the dried up abandoned sludge lagoon, some were detected (Table 27). Both E. histolytica and Giardia sp. appeared to be most prevalent in sludge 15 cm below the surface. With the drying of the sludge lagoon, parasites in the exposed sludge were apparently most susceptible. Viability of possible parasites was not tested but they appeared normal. Ascaris sp. was found only at the 15 cm depth and in low frequency. E. coli was observed relatively frequently at both sludge depths.

Substantial difficulty was encountered in looking for human parasitic nematodes in sludge because of the large numbers of freeliving and plant parasitic nematodes present. Eimeria sp. was the most prevalent parasite, but it may have come from cattle that had access to the sludge lagoon.

Detection of Nematode Larvae in Soil

Approximately one fourth of the larvae detected on the farm appeared to be in the genus Strongyloides, but we could not determine if they were human parasites (Table 28). The majority of the Strongyloides larvae were Strongyloid-like and were probably Haemonchus contortis. Generally, there appeared to be a higher density of these nematode larvae on the sewage farm than off. Figure 19 shows the sewage farm field locations that were sampled for nematodes.

The eggs and larval stages of these nematodes are susceptible to temperature and more importantly to drying. The soil on the sewage farm has been treated with wastewater over a long period of time which has increased the soil's organic matter and ability to retain moisture. This, along with keeping the soil relatively moist from frequent application of sewage wastewater, provides a favorable environment for larval nematode to survive. Thus, the nematodes escaped the natural ranges of humidity and precipitation that occur in the San Angelo area. It is also probable that the lusher vegetation on the sewage farm was beneficial to larval parasites by providing protection from direct sunlight.

Some difficulty in identifying parasitic larvae was encountered because of the large number of free living soil nematodes. Attempts to separate soil nematodes from parasitic forms by treating with different concentrations of HCl were not successful.

Detection of Parasites in Livestock Feces

Comparisons of parasite levels in fresh cattle (Tables 29, 30, 31, 32) and sheep feces (Table 33 and 34) collection on the sewage farm and off the sewage farm indicated a higher density of parasites on the sewage farm. Only the major genera of parasites observed were included in the tables,

TABLE 28. NUMBER OF STRONGYLOIDIDAE IN SURFACE SOIL FROM THE SEWAGE FARM AND A FARM NOT RECEIVING SEWAGE DURING NOVEMBER, DECEMBER AND JANUARY

Location	Sample Number	November	December	January[a]
		No/50cc soil		
Control Farm				
Field 1	1	3	0	3(18)
	2	23	0	
	3	--	0	
	4	--	0	
Sewage Farm				
Field 1	1	9	36	13(9)
	2	14	27	
	3	--	0	
	4	--	0	
Field 2	1	--	0	32(9)
	2	--	30	
	3	--	0	
	4	--	0	
Field 3	1	5	68	--
	2	2	0	--
	3	--	12	--
	4	--	12	--
Field 4	1	--	27	--
	2	--	0	--
	3	--	0	--
	4	--	15	--
Field 5	1	3	35	--
	2	52	0	--
	3	--	29	--
	4	--	0	--
Field 6	1	--	0	--
	2	--	26	--
	3	--	0	--
	4	--	0	--
Field 8	1	407	--	--
	2	0	--	--

[a]The data is an average and the number in parenthesis is the number of samples taken. Only 1 sample collected from the control farm was positive but 4 and 7 of the samples, respectively, from fields 1 and 2 on the sewage farm were positive.

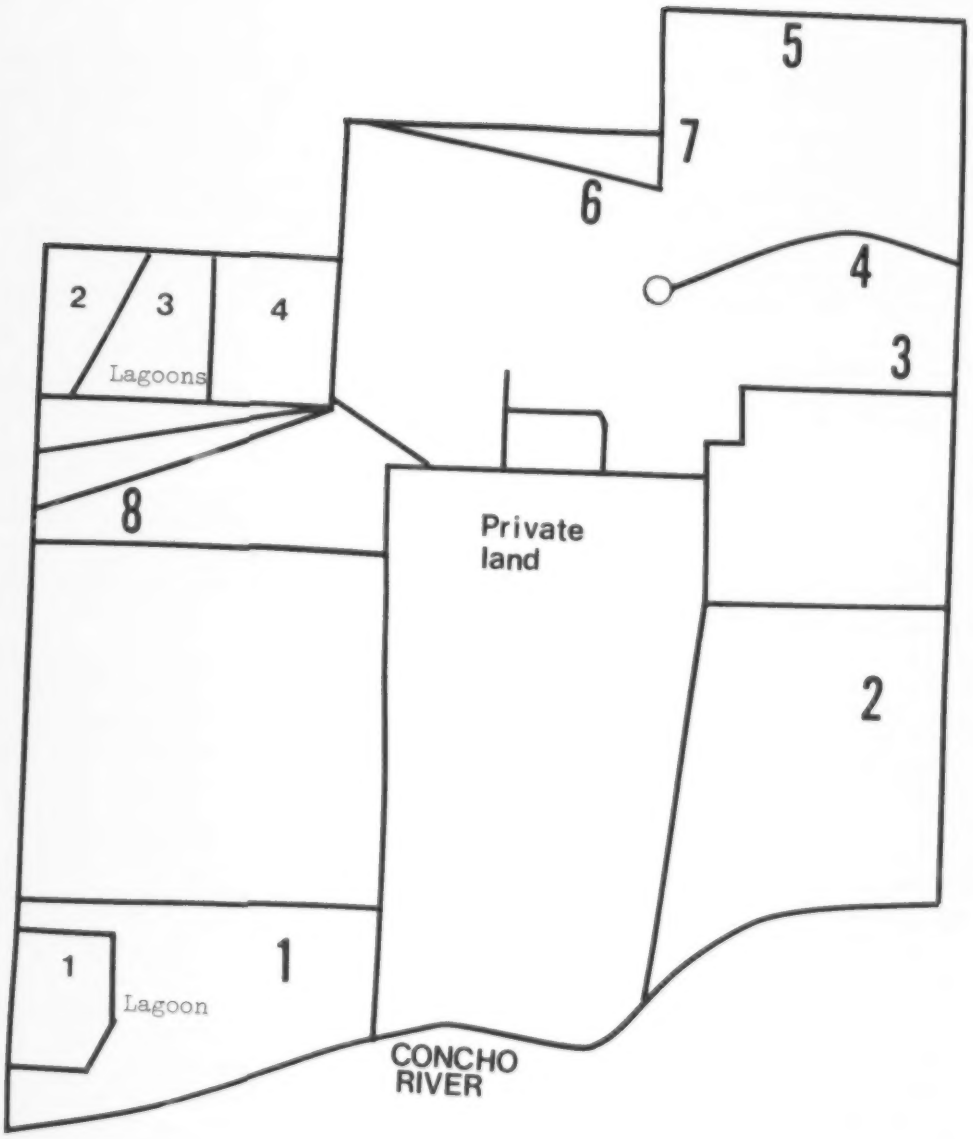

Figure 19. Diagram of sewage farm.

Approximate locations where soil and feces samples were taken, from the sewage farm, for detection of nematodes parasitic on animals.

but Nematodirus (nematode), Skrjabinema sp. (nematode), unidentified nematode eggs, unidentified oocysts (protozoan), and Entamoeba sp. (protozoan) were also observed. Figure 19 shows the locations of sewage farm fields listed in these tables.

Gonglyonema was found only on the sewage farm and detected in about one out of every five fecal samples (Table 29). This parasite was not detected off the sewage farm. Because the cattle on the sewage farm and those on the control area had different histories, we cannot be sure that the sewage application was the cause of the apparent higher incidence of Gongylonema on the sewage farm. However, Gongylonema nematode is reasonably widespread and both populations of cattle had likely been previously exposed to it. An adequate number of monthly samples was not taken off the sewage farm to make statistically valid comparisons.

In contrast to Gongylonema, the protozoan Eimeria was found in nearly all feces samples (Table 30). Eiremia is generally non-pathogenic unless present in very large numbers and is a common parasite of livestock.

The nematode Haemonchus is a detrimental parasite of cattle. This parasite seemed to be more prevalent in cattle on the sewage farm as compared to cattle off the sewage farm (Table 31), but an adequate number of monthly observations was not made on the control farm to allow for statistical comparisons. Egg counts were used as an indication of parasite burden and may not be valid for this parasite

To more adequately examine the difference between on-farm and off-farm levels of Eimeria and Haemonchus in cattle feces, nine samples were taken from each location on one occasion (Table 32). The results showed a significantly higher density of both parasites in cattle on the sewage farm.

The population of parasites in fecal samples from sheep appeared larger on the sewage farm (Table 33). However, too few monthly samples were taken for valid statistical comparison. Strongyloides was detected in two samples collected on the farm. This parasite was not detected in cattle feces.

As with the cattle, a one-time sampling of nine fecal samples from sheep was taken from the control farm and one field on the sewage farm. Eimeria and Haemonchus were much more numerous in the samples collected on the sewage farm (Table 34). Haemonchus was not detected in any fecal sample from sheep being grazed on the control farm not receiving sewage. The data collected demonstrate larger populations of parasites on the sewage farm, but does not prove that this was due to sewage application. The two groups of sheep may have had different histories of contact with parasites before arriving at the two farms.

Monitoring of Parasite Buildup in Cattle Feces

The previous experiment summarized in this report suggested that application of sewage effluent to pasture being grazed by cattle may have

TABLE 29. THE NUMBER OF GONGYLONEMA IN MANURE COLLECTED DURING 4 MONTHS FROM CATTLE BEING GRAZED ON THE SAN ANGELO SEWAGE FARM AND ON AN ADJACENT CONTROL FARM NOT RECEIVING SEWAGE

Location	Sample Number	September	October	November	December
		No. per g manure			
Control Farm					
Field 1	1	0	--	0	0
	2	0	--	--	0
	3	0	--	--	0
Sewage Farm					
Field 1	1	--	3	0	--
	2	--	3	0	--
Field 2	1	0	--	--	0
	2	0	--	--	0
	3	--	--	--	0
Field 3	1	--	--	0	0
	2	--	--	0	0
	3	--	--	--	0
	4	--	--	--	10
Field 4	1	--	3	--	0
	2	--	3	--	0
Field 5	1	0	0	--	0
	2	0	0	--	0
Field 6	1	--	--	--	0
	2	--	--	--	1
Field 7	1	--	--	0	--
	2	--	--	0	--
Field 8	1	--	--	0	--
	2	--	--	0	--

TABLE 30. THE NUMBER OF *EIMERIA* IN MANURE COLLECTED DURING 4 MONTHS FROM CATTLE BEING GRAZED ON THE SAN ANGELO SEWAGE FARM AND ON AN ADJACENT CONTROL FARM NOT RECEIVING SEWAGE

Location	Sample Number	September	October	November	December
		No. per/g manure			
Control Farm					
Field 1	1	2	--	6	4
	2	14	--	--	16
	3	3	--	--	3
Sewage Farm					
Field 1	1	--	13	TNTC[a]	--
	2	--	2	7	--
Field 2	1	3	--	--	[illegible]
	2	2	--	--	[illegible]
	3	--	--	--	6
Field 3	1	--	--	6	21
	2	--	--	3	90
	3	--	--	--	17
	4	--	--	--	24
Field 4	1	--	TNTC	--	25
	2	--	TNTC	--	24
Field 5	1	6	14	--	61
	2	12	21	--	33
Field 6	1	--	--	--	88
	2	--	--	--	64
Field 7	1	--	--	46	--
	2	--	--	29	--
Field 8	1	--	--	2	--
	2	--	--	0	--

[a]TNTC means too numerous to count

TABLE 31. THE NUMBER OF *HAEMONCHUS* IN MANURE COLLECTED DURING 4 MONTHS FROM CATTLE BEING GRAZED ON THE SAN ANGELO SEWAGE FARM AND ON AN ADJACENT CONTROL FARM NOT RECEIVING SEWAGE

Location	Sample Number	September	October	November	December
		No. per/g manure			
Control Farm					
Field 1	1	0	--	6	3
	2	2	--	--	0
	3	0	--	--	--
Sewage Farm					
Field 1	1	--	5	45	--
	2	--	5	6	--
Field 2	1	4	--	--	4
	2	0	--	--	5
	3	5	--	--	3
Field 3	1	4	--	1	266
	2	0	--	1	50
	3	2	--	--	159
	4	--	--	--	40
Field 4	1	--	56	--	3
	2	--	11	--	4
Field 5	1	0	28	--	10
	2	0	34	--	49
Field 6	1	--	--	--	0
	2	--	--	--	139
Field 7	1	--	--	5	--
	2	--	--	0	--
Field 8	1	--	--	1	--
	2	--	--	0	--

TABLE 32. THE NUMBER OF PARASITES, IN TWO GENERA, DETECTED IN CATTLE MANURE COLLECTED IN JANUARY ON THE SAN ANGELO SEWAGE FARM AND ON AN ADJACENT FARM NOT RECEIVING SEWAGE

Location	Number	Family	
		Eimeria	Hamonchus
		No. per/g feces	
Control Farm Field 1	1	3	1
	2	15	0
	3	20	0
	4	0	2
	5	0	11
	6	1	0
	7	0	0
	8	0	7
	9	7	0
Sewage Farm Field 1	1	11	47
	2	14	32
	3	19	21
	4	30	0
	5	11	273
	6	20	154
	7	17	20
	8	29	0
	9	35	15

TABLE 33. THE NUMBER OF PARASITES, IN THREE GENERA, DETECTED IN SHEEP MANURE COLLECTED DURING 3 MONTHS ON THE SAN ANGELO SEWAGE FARM AND ON AN ADJACENT FARM NOT RECEIVING SEWAGE

Genus	Month	Control Farm Sample		Sewage Farm Sample	
		1	2	1	2
		No. per/g feces			
Eimeria	Oct.	--	--	TNTC[a]	TNTC
	Nov.	6	0	0	4
	Dec.	1	8	19	19
Haemonchus	Oct.	--	--	1	0
	Nov.	6	0	131	0
	Dec.	2	0	311	137
Strongyloides	Oct.	--	--	5	2
	Nov.	0	0	0	0
	Dec.	0	0	0	0

[a]TNTC means too numerous to count

TABLE 34. THE NUMBER OF PARASITES, IN TWO GENERA, DETECTED IN SHEEP MANURE COLLECTED IN JANUARY ON THE SAN ANGELO SEWAGE FARM AND ON AN ADJACENT FARM NOT RECEIVING SEWAGE

Location	Sample Number	Family	
		Eimeria	Haemonchus
		No. per/g feces	
Control Farm Field 1	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
	6	5	0
	7	3	0
	8	1	0
	9	1	0
Sewage Farm Field 1	1	21	105
	2	18	94
	3	3	1
	4	34	4
	5	166	0
	6	118	37
	7	TNTC[a]	1
	8	TNTC	61
	9	32	52

[a]TNTC means too numerous to count

increased the population of parasites in the cattle. To better evaluate this hypothesis, a controlled study was designed in which cattle were monitored monthly for parasites, beginning the day the cattle were brought to the sewage farm. The number of Eimeria in the cattle did not increase with time on the sewage farm (Table 35), nor did the population of Haemonchus (Table 36).

Each animal was wormed with an antihelminthic drug when brought to the sewage farm. Yet no reduction in the population of parasites was observed after worming. Five of the ten cattle were wormed a second time with a noticeable reduction in parasites. Either the worming agent was not effective or the month interval between worming and taking the measurement was enough time for the parasite population to lower and increase to the previous levels.

The cattle in this experiment contained more parasites when brought onto the farm than did the cattle in the off-farm control areas (Table 32). Perhaps the method of collecting fecal samples was one reason for this difference. Fecal samples were taken directly from the cattle in this experiment, but were taken from droppings in the earlier experiments. The moisture and pH of manure changes rapidly after being voided by the animal.

TABLE 35. AVERAGE NUMBER OF EIMERIA SP. IN FECES FROM 10 TEST CATTLE FOR 7 MONTHS AFTER ARRIVING ON THE SEWAGE FARM

	Test Animals										Controls[a]	
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#1	#2
	No. per/g feces											
On Arrival	6	265	85	606	36	TNTC[b]	121	126	202	70	-	-
March	95	8	TNTC	9	120	11	22	TNTC	55	9	-	-
April	95	4	TNTC	9	120	11	22	TNTC	55	9	-	-
May	TNTC	7	30	31	98	TNTC	14	TNTC	TNTC	-	172	10
June	TNTC	34	TNTC	25	TNTC	98	68	99	52	81	39	3
July	12	8	126	1	185	88	13	TNTC	47	65	29	61
August	32	10	159	7	1	19	4	266	16	TNTC	TNTC	48

[a] These two cattle were already on the farm when the ten cattle arrived

[b] TNTC means too numerous to count

TABLE 36. AVERAGE NUMBER OF *HAEMONCHUS* SP. IN FECES FROM 10 TEST CATTLE FOR 7 MONTHS AFTER ARRIVING ON THE SEWAGE FARM

	Test Animals										Controls[a]	
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#1	#2
	No. per/g feces											
On Arrival	44	6	69	22	42	78	118	145	26	54	-	-
March	56	4	146	51	33	78	152	63	33	48	-	-
April	67	73	50	-	-	49	35	40	-	48	-	-
May	165	23	120	73	63	207	78	77	47	-	48	108
June	104	39	232	178	107	41	100	99	52	81	39	3
July	78	4	54	5	17	61	30	95	70	53	30	127
August	201	8	48	18	12	41	16	41	36	30	28	1

[a]These two cattle were already on the farm when the ten cattle arrived

SECTION 7

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